Contents

Original Articles

Case reports

Review Articles

Short Communications

Proceedings of Paediatric Societies

Supplements

Case reports

Review Articles

Short Communications

Proceedings of Paediatric Societies

Supplements

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RELATIONSHIP BETWEEN GLOMERULAR FILTRATION RATE AND RADIOLOGICAL APPEARANCE OF THE RENAL PARENCHYMA IN CHILDREN

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The present study is an attempt to reveal the functional significance of renal parenchymal changes as demonstrated by IVP s The patients included in this study were generally referred to the radiological examination because of the history of one or more urinary tract infections The study was necessitated by (1) the old observation of an often poor correlation between clinical history and radiological finding (2) the inevitable question in case of significant differences in kidney size shall nephrectomy of the small kidney be carried out and will that abolish the source of infection?

The functional evaluation has in the present report been limited to the glomerular filtration rate[1] as determined by the clearance of inulin In order to fulfil the outlined intentions selective renal studies were carried out This was accomplished by external ureteral compression Since this method has not been used previously in childhood practice this report will also include a methodological evaluation

MATERIAL

The material includes 33 patients aged 3 to 15 years All those patients except two have at one time or another had clinical signs of urinary tract infection The patients did not have any clinical or bacteriological signs of infection at least 2 months preceeding the study

For radiological classification the patients have been grouped on the basis of the intravenous pyelography (IVP) The radiological studies which included an IVP as well as a micturition cystogram generally preceeded the functional studies by 1 to 6 months For various reasons the main purpose of this work has been to evaluate the function of the "radiological small kidney" Group I (Table 1) therefore consists of patients where the IVP has demonstrated one kidney that has been significantly small in relation to the age of the patient. The size of the kidney has been such that the question has been raised whether the function of this kidney could be more than 20% of the total renal function Thus all of the patients have been referred to nephrectomy The clinical motivation for this was to abolish the source of infection Fig 1 including all the patients in group I demonstrate the lateral shape of the small kidney as compared with the lateral shape of a normal kidney of a subject in the corresponding age group and sex No attempt has been made for a more precise calculation of the kidney size since the methods presently used are controversial and very rough In only one of the patients in group I L. N was there any sign of more than slight parenchymal reduction on the contralateral side

As a reference two additional groups of patients have been studied. The control group II (Table 2) consists of patients with a normal IVP Two of these patients had no history of urinary tract infection, while the third L. J was considered to have had one prolonged infection with symptoms mainly of cystitis. Group III (Table 3) consists of patients where the IVP shows scarring of one or both kidneys. The parenchymal reduction was however so small that it was not considered of any importance for renal function

In addition to the radiological classification an attempt has been made to group the patients accord

Supported by Swedish Medical Research Council Grant B 67 19 x 2049-01A

Abbreviation. Glomerular filtration rate = GFR

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In addition to the radiological classification an attempt has been made to group the patients accord

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Abbreviation Glomerular filtration rate = GFR

Table 1 (*cont*)

Patient and birth day	Group	Clinical history	Radiological finding: Small kidney	Radiological finding: Contralateral kidney	Glomerular filtration rate ml/min/1.73 m²: Small kidney	Contralateral kidney	Small kidney in % of contralateral kidney	Small kidney in % of total renal function
			IVP					
K G female 630930	B	Bacteriuria 1 yearly. Moderate symptoms. Prompt treatment	Small with somewhat reduced contrast density. Deformation of calices	Renal parenchyma normal in size. No scarring. 2 pelvis. Some of the calices deformed	42	41	102	51
			Micturition cystogram					
			Large reflux	Reflux				
			IVP					
I L L female 580123	B	First year of age poor weight gain. No urine analysis made. Later asymptomatic till the age of 6 when bacteriuria with slight symptoms once yearly	Small kidney. Deformation of calices, no papillas	Normalized kidney with good concentration of contrast medium. Slight "scar" in the lower part	35	23	148	60*
			Micturition cystogram					
			Large bladder. No reflux	Moderate reflux				
			IVP					
I A female 580178	C	First symptomatic infection with bacteriuria at the age of 2½. High relapse frequency 3–5 yearly despite intensive antibiotic treatment	Small kidney with good concentration of the contrast medium. Deformation of some of the calices	Normalized kidney with good concentration of contrast medium. Slight scar in the lower part	20	73	27	22
			Micturition cystogram					
			Moderate reflux	Moderate reflux				
			IVP					
E M L female 6309..	C	Since the age of 2 2–3 infections with bacteriuria, fever and left sided abdominal pain radiating dorsally	Very small kidney with even contour and good concentration of contrast medium. Deformation of calices	Normal	24	83	29	22
			Micturition cystogram					
			Moderate reflux	Large reflux				

Table 1 *Group I Small kidney*

Patient and birth day	Group	Clinical history	Radiological finding: Small kidney	Radiological finding: Contra lateral kidney	Glomerular filtration rate ml/min/1 73 m^2: Small kidney	Contra lateral kidney	Small kidney in % of contra lateral kidney	Small kidney in % of total renal function
			IVP					
C L male 531215	A	Clinical and bacterial evidence of only one urinary tract infection (age 12 years)	Very small but with good and even concentra tion of the con trast medium	Normal	54	55	98	50
			Micturition cystogram					
			No reflux	No reflux				
			IVP					
G B female 600706	B	Since the age of 3 years bac teriuria once yearly Prompt treatment Slight symptoms No fever no abdominal pain	Very small kidney with thin paren chyma and deformation of the calices [b]	Normal or slightly hypertro phied kidney	33	78	42	30
			Micturition cystogram					
			Large reflux	Large reflux				
			IVP					
B S female 610320	B	Since the age of 8 months bacteriuria 1–2 yearly Prompt treat ment Occasional ly fever and rightsided abdo minal pain	Small kidney with signs of deformation in some of the calices [b]	Normal kidney	33	79	41	29
			Micturition cystogram					
			Large reflux with moderate dilatation	Large reflux with moderate dilatation				
			IVP					
E B female 640425	B	Since the age of 2 months bacteriuria 1–2 yearly Prompt treat ment No fever no abdominal pain	Small kidney with good concentration of contrast medium in the thin parenchyma Slightly deformed calices	Normal kidney	20	54	38	27
			Micturition cystogram					
			Moderate reflux	Normal				
			IVP					
K M male 621115	B	Bacteriuria 1 yearly	Small kidney with good concentration of contrast medium in the renal paren chyma	Normal sized with good concentration of contrast medium No actual scarring but slight waist line	17	25	67	40[c]
			Micturition cystogram					
			Moderate reflux	Normal				

Table 1 (cont.)

Patient and birth day	Group	Clinical history	Radiological finding: Small kidney	Radiological finding: Contralateral kidney	Glomerular filtration rate ml/min/1.73 m²: Small kidney	Contralateral kidney	Small kidney in % of contralateral kidney	Small kidney in % of total renal function
			IVP					
K G female 630930	B	Bacteriuria 1 yearly Moderate symptoms Prompt treatment	Small with somewhat reduced contrast density Deformation of calices	Renal parenchyma normal in size No scarring. 2 pelvis Some of the calices deformed	42	41	102	51
			Micturition cystogram					
			Large reflux	Reflux				
			IVP					
I L L female 580123	B	First year of age poor weight gain No urine analyses made Later asymptomatic till the age of 6 when bacteriuria with slight symptoms once yearly	Small kidney Deformation of calices no papillas	Normalsized kidney with good concentration of contrast medium. Slight "scar" in the lower part	35	23	148	60*
			Micturition cystogram					
			Large bladder No reflux	Moderate reflux				
			IVP					
I A female [illegible]	C	First symptomatic infection with bacteriuria at the age of 2½ High relapse frequency 3–5 yearly despite intensive antibiotic treatment	Small kidney with good concentration of the contrast medium Deformation of some of the calices	Normalsized kidney with good concentration of contrast medium Slight "scar" in the lower part	20	73	27	22
			Micturition cystogram					
			Moderate reflux	Moderate reflux				
			IVP					
E M L female 630922	C	Since the age of 2 2–3 infections with bacteriuria fever and left sided abdominal pain radiating dorsally	Very small kidney with even contour and good concentration of contrast medium Deformation of calices	Normal	24	83	29	22
			Micturition cystogram					
			Moderate reflux	Large reflux				

Table 1 (*cont*)

Patient and birth day	Group	Clinical history	Radiological finding: Small kidney	Radiological finding: Contra lateral kidney	Glomerular filtration rate ml/min/1 73 m²: Small kidney	Contra lateral kidney	Small kidney in % of contra lateral kidney	Small kidney in % of total renal function
			IVP					
S W female 631217	C	Since the age of 1 year 5–10 infections with bacteriuria yearly Fever occasionally	Small kidney Calices deformed ᵇ	Normal	24	68	36	27
			Micturition cystogram					
			Large reflux	No reflux				
			IVP					
L N female 581222	C	Since at least the age of 3 years frequent infections with bacteriuria Fever occasionally	Significantly reduced parenchyma Uneven contour Deformed calices ᵇ	Slightly reduced parenchyma Uneven contour Deformed calices ᵇ	41	42	99	50
			Micturition cystogram					
			Moderate reflux	No reflux				

Slight symptoms frequent micturitions (without polyuria) and dysuria
ᵇ The expression deformation of calices includes irregularities in arrangement and shape of the calices the lack or flattening of the papillas
Study performed under general anesthesia

ing to the clinical history This grouping has been made particularly with regard to the estimated relapse frequency For each group the following criteria have been required

Group A Clinical history of at most one infection with or without significant bacteriuria

Group B Clinical history of one to three urinary tract infections yearly All those patients have had bacteriuria at the time of the symptoms In none of the patients have there been recurrent episodes of high fever or high abdominal pain radiating dorsally

Group C Clinical history of three or more infections yearly confirmed by bacteriuria or recurrent episodes of high fever and one sided abdominal pain radiating dorsally and simultaneous bacteriuria

Group I consists of 11 patients with one patient in group A namely C L with one infection with significant bacteriuria The rest of the clinical history of this patient is completely noncontributory Six patients of group I are included in group B and four patients in group C In group C patients I A S W and L N have had more than three infections yearly One patient E M L has had recurrent episodes of high fever and leftsided abdominal pain radiating dorsally and simultaneous bacteriuria

In group II are included two patients without any signs of urinary tract infection One of those patients were admitted because of proteinuria (S N) one because of hypertension (M J) and the third patient (L J) has had one prolonged urinary tract infection with clear symptoms of cystitis

Group III consists of six patients None of those fulfil the criteria of group A Three patients belong to group B and three to group C

METHODS

On the day before the study the patients received one to several enemas On the day of the study they were given a standard breakfast meal and were allowed free water intake Standard clearance technic was used (20) This includes a continuous infusion of 10 inulin (Laevasan Gesellschaft) 0 001 g/min/kg b w after the prime dose of 0 05 g inulin/kg b w The continuous infusion was started at least 1 hour before the study For urine sampling the bladder was catheterized with a double lumen polyethylene catheter enabling a continuous suction Blood samples were obtained in the middle of each urine collection period from an indwelling needle in a peripheral vein To ensure a high diuresis the patients were given a

constant infusion of 20 $_{\%}$ glucose Glucose was chosen instead of mannitol since later studies which are now in progress intend to determine the T_m glucose in this material

Unilateral clearance studies were accomplished in the following way External ureteral compression technic was used as described by Bernstein & Hamby (1) The pressure was applied on the anterior abdominal wall pressing the ureter against the posterior brim of the pelvic inlet The complete ureteral occlusion by this technic as well as the adequacy of urine sampling, was continuously checked by means of high amplifying fluoroscopy For this purpose an intravenous injection of 60 Urografin (Schering) 0 5–1 ml/kg b w was given 5 minutes before compression The duration of the ureter compression never exceeded 15 minutes

The chemical analyses of inulin in serum and urine were carried out according to the method of Heyrowski (11) Before the analyses 0 09 g baker s yeast (0 3 ml of a 30% yeast suspension) was added to the samples (0 2 ml plasma) in order to eliminate the interference with glucose (3)

EVALUATION OF METHOD

The validity of the technic is highly dependent on adequate urine sampling Since the unilateral studies necessarily will be of short duration inadequate urine sampling would constitute a proportionately large error of method Ureteral reflux might be one possible complication in bladder emptying Since a rather high percentage of our patients have refluxes of varying degree (see Table 1 and 3) this complication must be excluded The method has therefore been evaluated in three groups of patients namely (1) patients without signs of ureteral reflux (2) patients with moderate ureteral reflux but without signs of dilatation of ureter and/or renal pelvis and finally (3) patients with large ureteral reflux with signs of dilatation of ureter and/or renal pelvis Fig 2 (*a* and *b*) demonstrates typical micturition cystograms from two patients with moderate and severe reflux respectively

Tables 4 to 6 give the GFR in the three groups Some of the patients in these tables are not included in the later tables namely those with unilateral or bilateral hydronephrosis These patients are excluded in the further report because hydronephrosis might influence

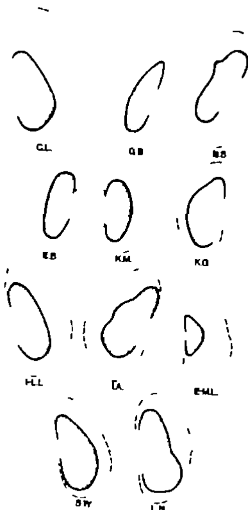


Fig 1 The lateral shape of the "small" kidney of all the patients in group I (thick line) The dashed line represents the size of a normal kidney from a healthy patient of the same age

renal function However for methodological evaluation hydronephrosis is of no significance In each patient in the tables the average values from two unilateral clearance determinations are given These values have then been added (i e the calculated bilateral glomerular filtration rate) and compared to the results from the bladder sampling before ureteral compression i e the found bilateral values The found bilateral values represent the mean of two to four clearance periods The individual scatter between those periods does not exceed 20%. The tables demonstrate the correlation coeffi

Table 2 *Group II Normal kidney*

Patient and birth day	Group	Clinical history	Radiological finding: Small kidney	Radiological finding: Contra lateral kidney	Glomerular filtration rate ml/min/1 73 m²: Small kidney	Contra lateral kidney	Small kidney in % of contra lateral kidney	Small kidney in % of total renal function
			IVP					
M J male 620112	A	Hypertension of nonrenal origin (hyper aldosteronism) known since 66	Normal	Normal	50	52	96	
			Micturition cystogram					
			Normal	Normal				
			IVP					
S N female 560925	A	Proteinuria known since 65 No signs of nephro is	Normal	Normal	48	49	96	
			Micturition cystogram					
			Normal	Normal				
			IVP					
L J female 590710	A	One infection with bacteriuria 64 Slight symptoms [a]	Normal	Normal	72	62	116	
			Micturition cystogram					
			Normal	Normal				

Table 3 *Group III Scarred kidney*

Patient and birth day	Group	Clinical history	Radiological finding: Small kidney	Radiological finding: Contra lateral kidney	Glomerular filtration rate ml/min/1 73 m²: Small kidney	Contra lateral kidney	Small kidney in % of contra lateral kidney	Small kidney in % of total renal function
			IVP					
I M K female 550420	B	Since the age of 1 year in fections with bacteriuria 2–3 yearly Fever occa sionally	Moderately reduced parenchyma Deformation of calices [b]	Slightly reduced scarred parenchyma Some of the the calices deformed	35	80	44	30
			Micturition cystogram					
			No reflux	No reflux				
			IVP					
C N female 640720	B	Since the age of 1 year in fections with bacteriuria 1–2 yearly Slight symp toms	Slight parenchymal reduction in the lower part Calices normal	Normal	57	53	107	52
			Micturition cystogram					
			Large reflux	No reflux				

Table 3 (*cont.*)

Patient and birth day	Group	Clinical history	Radiological finding: Small kidney	Radiological finding: Contralateral kidney	Glomerular filtration rate ml/min/1.73 m²: Small kidney	Contralateral kidney	Small kidney in % of contralateral kidney	Small kidney in % of total renal function
J. J. male 580531	B	61–64 1–3 infections yearly with bacteriuria, fever and abdominal pain occasionally. Since 64 no clinical or bacteriological signs of infection	IVP: Scarred kidney with moderately reduced parenchyma. Small calcifications in upper part. One calice (in upper part) deformed.* Micturition cystogram: Moderate reflux	IVP: Normal-sized parenchyma. Slight calcifications in upper part Micturition cystogram: Slight–moderate reflux	22	47	46	32
A. B. female 560629	C	Since the age of 5 years 2–4 infections yearly, generally with fever and abdominal pain radiating dorsally	IVP: Moderate parenchymal reduction. Very uneven contour. Several small calcifications Micturition cystogram: Moderate reflux	IVP: Normal Micturition cystogram: No reflux	23	60	38	28
C. K. female 5703.0	C	Since the age of 4 years 4–5 infections with bacteriuria yearly. Fever occasionally	IVP: Slight parenchymal reduction in lower part. Calices Normal Micturition cystogram: Slight reflux	IVP: Normal Micturition cystogram: No reflux	46	43	109	52
K. C. female 571028	C	Since the age of 4 years 2–3 infections yearly with bacteriuria. Symptoms generally fevers with acute stress fever, abdominal pain	IVP: Slight scarring. Without actual parenchymal reduction Micturition cystogram: Large reflux	IVP: Normal Micturition cystogram: No reflux	49	70	70	41

Slight symptoms include frequent micturitions (without polyuria) and dysuria.
* The expression deformation of calices includes irregularities in arrangement and shape of the calices, the lack or flattening of the papillas.

Table 2 *Group II Normal kidney*

Patient and birth day	Group	Clinical history	Radiological finding: Small kidney	Radiological finding: Contra lateral kidney	Glomerular filtration rate ml/min/1 73 m²: Small kidney	Contra lateral kidney	Small kidney in % of contra lateral kidney	Small kidney in % of total renal function
			IVP					
M J male 620112	A	Hypertension of nonrenal origin (hyper aldosteronism) known since 66	Normal	Normal	50	52	96	
			Micturition cystogram					
			Normal	Normal				
			IVP					
S N female 560925	A	Proteinuria known since 65 No signs of nephro is	Normal	Normal	48	49	96	
			Micturition cystogram					
			Normal	Normal				
			IVP					
L J female 590710	A	One infection with bacteriuria 64 Slight symptoms	Normal	Normal	72	62	116	
			Micturition cystogram					
			Normal	Normal				

Table 3 *Group III Scarred kidney*

Patient and birth day	Group	Clinical history	Radiological finding: Small kidney	Radiological finding: Contra lateral kidney	Glomerular filtration rate ml/min/1 73 m²: Small kidney	Contra lateral kidney	Small kidney in % of contra lateral kidney	Small kidney in % of total renal function
			IVP					
I M K female 550420	B	Since the age of 1 year infections with bacteriuria 2–3 yearly Fever occasionally	Moderately reduced parenchyma Deformation of calices *	Slightly reduced scarred parenchyma Some of the calices deformed *	35	80	44	30
			Micturition cystogram					
			No reflux	No reflux				
			IVP					
C N female 640720	B	Since the age of 1 year infections with bacteriuria 1–2 yearly Slight symptoms	Slight parenchymal reduction in the lower part Calices normal	Normal	57	53	107	52
			Micturition cystogram					
			Large reflux	No reflux				

Table 6 *The calculated and found GFR in patients with large reflux*

GFR (ml min/1 73 m² b s)

Patient	Right kidney GFR	Left kidney GFR	The calculated bilat GFR	The found bilat GFR
GB	33	78	111	106
HE	96	96	192	183
EK	76	46	122	122
EML	83	24	107	93
BS	33	79	112	111
SW	68	24	92	85
KG	70	49	119	121
CN	53	57	110	106
KO	42	41	83	84

0 986
$t = 15\ 3$
$p < 0\ 01$

discrepancy between paired samples is also somewhat larger Group 3 Table 6 consists of patients with large ureteral reflux and secondary dilatation of the ureter and/or renal pelvis It is remarkable that the correlation coefficient between estimated and experimentally found bilateral GFR is as high as 0 986 In addition the difference between paired samples is lower than 5 in 7 of 9 patients Thus ureteral reflux of varying degree seems little to influence the adequacy of urine sampling

The method with external ureteral compression technic is apparently of special value in a more detailed study of unilateral renal function in childhood The other alternative of making selective renal clearance studies namely a method involving ureteral catheterization will be less valid in childhood practice The two main reasons for this are (1) the size of the pediatric cystoscope will not allow the passage of sufficiently large catheters supplied with appropriate cuffs (2) even if a sufficiently large catheter could be introduced in some of the patients a right ureteral fit around the catheter can only be expected in patients without ureteral reflux The extremely good validity of the external ureteral compression technic has been demonstrated previously by Bernstein & Hamby (1 9 10) Those results have been confirmed in the present work Furthermore this study also demonstrates the complete validity of this method even in cases with moderate or severe reflux (correlation coefficient of 0 958 and 0 986) This finding is of special value not only for the present study but also for the evaluation of the total clearance technic in children with varying degrees of refluxes It should however be noted that the evaluation of this method has been made under the condition of rather heavy osmotic diuresis It is still possible that studies under hydropenia can be combined with rather high methodological error

RESULTS

GFR in group I

All the GFR values are expressed *in* ml/min/1 73 m body surface In only 5 out of 11 patients is the GFR of the small kidney lower than 25 ml/min i e the estimated 50 reduction from the normal value for adults (20) The lowest recorded GFR was 17 ml/min It is however to be noticed that this study was performed under general anesthesia In 5 of the 11 patients the GFR of the small kidney is between 30 and 42 ml/min/1 73 m The function of this kidney is probably good enough for the patient to escape renal insufficiency In patient C L the GFR is normal

In the next to last column Table I the GFR of the small kidney has been given in of the GFR of the contralateral kidney In 4 of 11 patients the GFR of the small kidney exceeds 98 of the contralateral kidney and in none of the patients is the GFR of the small kidney lower than 27 of the contralateral kidney Thus there appears to be little correlation between the IVP finding and the functional finding

If on the other hand renal function is correlated not to radiological size but rather to the clinical history with number and severity of urinary tract infections i e the grouping A *to* C a certain correlation appears to exist Thus the highest discrepancy in GFR between the

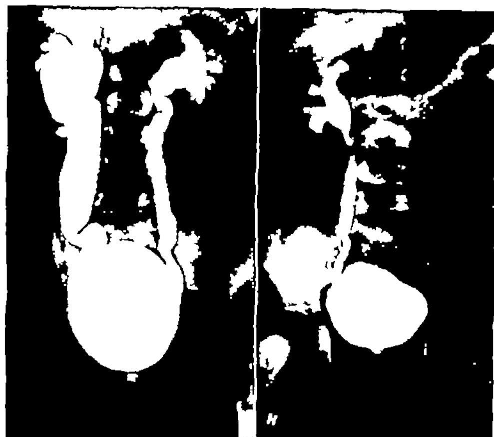


Fig 2 (a and b) Micturition cystogram from patient B S group I Table I and from patient E B group I Table I

cient and its *t* value In group I Table 4 namely the patients without radiological signs of ureteral reflux the correlation coefficient is 0 992 It should also be noticed that the difference between paired samples rarely exceeds 5 In group 2 Table 5 namely the patients where moderate ureter reflux has been demonstrated on the micturition cystogram the correlation coefficient is still highly significant but somewhat lower namely 0 958 In addition the

Table 4 *The calculated and found GFR in patients without signs of ureteral reflux*

GFR (ml/min/1 73 m² b s)

Patient	Right kidney GFR	Left kidney GFR	The calculated bilat GFR	The found bilat GFR
LE	14	58	72	77
CG	49	39	88	88
JH	22	22	44	40
MS	77	56	133	128
AK	40	7	47	49
SEK	74	22	96	102
FP	66	86	152	151
SN	48	49	97	100
MJ	50	52	102	94
IMK	35	80	115	118

$r = 0.992$ Study performed under general anesthesia
$t = 22.2$
$p < 0.01$

Table 5 *The calculated and found GFR in patients with moderate reflux*

GFR (ml/min/1 73 m² b s)

Patient	Right kidney GFR	Left kidney GFR	The calculated bilat GFR	The found bilat GFR
AKB	60	39	99	97
CE	75	49	124	109
WF	42	34	76	90
CK	43	46	89	95
ILL	23	35	58	42
KM	25	17	42	40
AB	60	23	83	84
ACB	13	20	33	29
ILG	41	85	126	139
IA	20	73	93	91
EB	20	54	74	67
LN	42	41	83	80
JJ	22	47	69	69

$r = 0.958$ Study performed under general anesthesia
$t = 11.0$
$p < 0.01$

Table 6 *The calculated and found GFR in patients with large reflux*

GFR (ml/min/1 73 m² b s)

Patient	Right kidney GFR	Left kidney GFR	The cal culated bilat GFR	The found bilat GFR
GB	33	78	111	106
HE	96	96	192	183
EK	76	46	122	122
EML	83	24	107	93
BS	33	79	112	111
SR	68	4	9	85
KG	70	49	119	121
CN	53	57	110	106
KO	42	41	83	84

$r = 0\ 986$
$t = 15\ 3$
$p < 0\ 01$

discrepancy between paired samples is also somewhat larger Group 3 Table 6 consists of patients with large ureteral reflux and secondary dilatation of the ureter and/or renal pelvis It is remarkable that the correlation coefficient between estimated and experimentally found bilateral GFR is as high as 0 986 In addition the difference between paired samples is lower than 5 in 7 of 9 patients Thus ureteral reflux of varying degree seems little to influence the adequacy of urine sampling

The method with external ureteral compression technic is apparently of special value in a more detailed study of unilateral renal function in childhood The other alternative of making selective renal clearance studies namely a method involving ureteral catheterization will be less valid in childhood practice The two main reasons for this are (1) the size of the pediatric cystoscope will not allow the passage of sufficiently large catheters supplied with appropriate cuffs (2) even if a sufficiently large catheter could be introduced in some of the patients a tight ureteral fit around the catheter can only be expected in patients without ureteral reflux The extremely good validity of the external ureteral compression technic has been demonstrated previously by Bernstein & Hamby (1 9 10) Those results have been confirmed in the present work Furthermore this study also demonstrates the complete validity of this method even in cases with moderate or severe reflux (correlation coefficient of 0 958 and 0 986) This finding is of special value not only for the present study but also for the evaluation of the total clearance technic in children with varying degrees of refluxes It should however be noted that the evaluation of this method has been made under the condition of rather heavy osmotic diuresis It is still possible that studies under hydropenia can be combined with rather high methodological error

RESULTS

GFR in group I

All the GFR values are expressed in ml/min/1 73 m body surface In only 5 out of 11 patients is the GFR of the small kidney lower than 25 ml/min i e the estimated 50 reduction from the normal value for adults (20) The lowest recorded GFR was 17 ml/min It is however to be noticed that this study was performed under general anesthesia In 5 of the 11 patients the GFR of the small kidney is between 30 and 42 ml/min/1 73 m The function of this kidney is probably good enough for the patient to escape renal insufficiency In patient C L the GFR is normal

In the next to last column Table I the GFR of the small kidney has been given in of the GFR of the contralateral kidney In 4 of 11 patients the GFR of the small kidney exceeds 98 of the contralateral kidney and in none of the patients is the GFR of the small kidney lower than 27 of the contralateral kidney Thus there appears to be little correlation between the IVP finding and the functional finding

If on the other hand renal function is correlated not to radiological size but rather to the clinical history with number and severity of urinary tract infections i e the grouping A to C a certain correlation appears to exist Thus the highest discrepancy in GFR between the

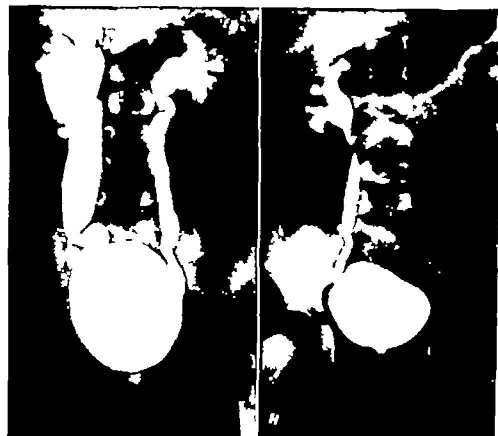


Fig 2 (*a* and *b*) Micturition cystogram from patient B S group I Table I and from patient E B group I Table I

cient and its *t* value In group I Table 4 namely the patients without radiological signs of ureteral reflux the correlation coefficient r 0 992 It should also be noticed that the difference between paired samples rarely exceeds 5% In group 2 Table 5 namely the patients where moderate ureter reflux has been demonstrated on the micturition cystogram the correlation coefficient r still highly significant but somewhat lower namely 0 958 In addition the

Table 4 *The calculated and found GFR in patients without signs of ureteral reflux*

GFR (ml/min/1 73 m² b s)

Patient	Right kidney GFR	Left kidney GFR	The calculated bilat GFR	The found bilat GFR
LE	14	58	72	77
CG	49	39	88	88
JH	22	22	44	40*
MS	77	56	133	128
AK	40	7	47	49*
SEK	74	22	96	102
FP	66	86	152	151
BN	48	49	97	100
MJ	50	52	102	94
IMK	35	80	115	118

$r = 0.992$
$t = 22.2$
$p < 0.01$

* Study performed under general anesthesia

Table 5 *The calculated and found GFR in patients with moderate reflux*

GFR (ml/min/1 73 m b s)

Patient	Right kidney GFR	Left kidney GFR	The calculated bilat GFR	The found bilat GFR
AKB	60	39	99	97
CE	75	49	124	109
WF	42	34	76	90
CK	43	46	89	95
ILL	23	35	58	42*
KM	25	17	42	40
AB	60	23	83	84
ACB	13	20	33	29
ILG	41	85	126	139
IA	20	73	93	91
EB	20	54	74	67
LN	42	41	83	80
JJ	22	47	69	69

$r = 0.958$
$t = 11.0$
$p < 0.01$

Study performed under general anesthesia

of renal parenchyma could vary At the present time two main conditions predisposing to renal parenchymal reduction in children (without the clinical history of glomerulonephritis) are recognized namely (1) recurrent pyelonephritis with consequent scarring (12 17 18 22) and (2) primary renal dysplasia (2 7 8 14 15 16) Since renal dysplasia predisposes to infection the pathology of some of the kidneys studied might be a mixture of both conditions The determinations of GFR only does not permit any further differential diagnosis between the two conditions On the basis of the apparently good inverse correlation between the glomerular filtration rate and the relapse frequency it might however be suggested that pyelonephritic scarring would relatively more damage the nephron than would renal dysplasia

One of the most surprising findings of this study was the generally relatively good and in some cases apparently normal function of some of the small kidneys From what has been described by Templeton *et al* (23) it is rather likely that the dimension of the main renal artery of those small kidneys *is reduced at least* to some extent It is therefore unlikely that total blood flow and vascular resistance would be exactly the same as in a normal kidney of a much larger size It has however been suggested by other investigators (3) that during normal conditions only part of the nephron population is functioning and the rest constitutes a reserve capacity It might therefore be suggested that in the case of the small kidney a much larger proportion of the nephron population is functioning with consequent reduction of the reserve capacity

The other extreme in the discrepancy between radiological and functional finding namely a relatively large kidney with proportional low GFR is just as noteworthy from the clinical point of view This finding was most pronounced in patients J J and A B in group III Table 3 and K G in group I Table 1 (contralateral kidney) Patients L N (contralateral side) in group I Table 1 and C K (contralateral side) in group III Table 3 have 20 reduction in function while the parenchymal reduction judged from the IVP could be estimated to at most 5% Since the total function in all the patients well exceeded 50% of normal the reduction in GFR could not be expected to be reflected in the usual screening tests i e serumcreatinine and urea The reason for the disproportionately large reduction in GFR in patients J J A B K G L N and C K cannot be defined on the basis of the present results but deserve more detailed renal function studies

SUMMARY

Selective renal function studies with determination of the glomerular filtration rate (inulin clearance) have been carried out in more than 30 children aged 3 to 15 years Most of the patients had a history of one or more urinary tract infections as well as pathological IVP findings of varying degree

The selective studies were accomplished by means of external ureteral compression The method proved to have an extremely good validity even in cases of severe ureteral reflux with dilatation of ureter and pelvis

No correlation could be found between radiological size of the kidney and glomerular filtration rate In almost half of the patients with significant unilateral reduction of the renal parenchyma *the* glomerular filtration rate of this side was found to be more than 40% of the total glomerular filtration rate In contrast the glomerular filtration rate of several of the kidneys estimated to be of normal size was significantly reduced It is obvious from these findings that nephrectomy must never be carried out until selective renal function studies have been made

An additional finding was an apparently good correlation between the clinical and functional finding i e the glomerular filtration rate appeared to be inversely correlated to the relapse frequency of the urinary tract infections The fact that the functional finding correlated well with the clinical finding but not with the

two kidneys is found in group C, i e the patients with the most severe and frequent relapses Also in group B i e the patients with moderate severe infections, there is a certain but less pronounced discrepancy in GFR between the two kidneys On the other hand the patient in group A who presented with the history of only one urinary tract infection was found to have a normal GFR on both sides, thus also of the small kidney

GFR in group II

Group II consists of patients with normal IVP s and with one exception without any history of urinary tract infection The average GFR of one kidney is 55 ml/min/1 73 m This value corresponds rather well to the reported normal values for the adults (20)

GFR in group III

Group III consists of patients with scarred kidneys Naturally the scarring was not completely symmetrical Thus one kidney was always somewhat more scarred than the other one The result of this kidney is presented in the "small kidney" column In 3 of the 6 patients the GFR of both kidneys exceeded 40 ml/min/1 73 m In the next to last column the function of the two kidneys is compared In 4 of the 6 patients the GFR of the most scarred kidney is significantly lower than the GFR of the contralateral side In 2 of the patients the GFR is approximately the same in the two kidneys Thus again no correlation can be demonstrated between radiological and functional finding In contrast to group I the correlation between clinical finding and functional finding also seems poor among those patients Thus 2 of 3 of the patients in group C, i e patients with high relapse frequency have good function on both sides (C K and K G)

DISCUSSION

The immediate implication of this study is the absolute necessity of selective renal function determinations before nephrectomy is carried out The two main indications for nephrectomy in children with recurrent urinary tract infections and unilateral reduction of the renal parenchyma are (1) renal hypertension and (2) chronic pyelonephritis more or less resistent to antibiotic treatment It is obvious from group I Table 1 that in most of those patients *nephrectomy would be contraindicated*

The validity of these indications for unilateral nephrectomy can be seriously questioned Renal hypertension is seldom due to outflux of hypertensive substances from one single kidney In most cases selective renal studies will show that both kidneys are damaged with regard to renal circulation Chronic pyelonephritis cannot be regarded as a more valid indication for nephrectomy It would indeed be optimistic to expect a chronic urinary tract infection of long standing to leave one kidney intact In addition there is at present great controversy on what is the primary source of infection A defect in the defence against ascending infections i e a defective resistance of the urinary bladder may be of primary importance (4 5 19 26)

Thus there is right now no clearcut indication for nephrectomy in pediatric patients with nephropathies other than tumors On the contrary the present work has demonstrated that in most cases nephrectomy is contraindicated In none of the patients presented was the renal function of the small kidney less than 20% of the total renal function and in 6 of the patients the function was more than 40% of the total renal function Another factor contributing to the contraindications towards nephrectomy is the commonly found functional reduction of the contralateral kidney It is hardly likely that a kidney with an already reduced renal function would be able to hypertrophy and increase its function beyond what is normal for one kidney

The lack of correlation between radiological and functional finding deserves a few comments One likely reason for this is the fact that the pathological basis for the reduction

THE VENOUS PLASMA FREE AMINO ACID LEVELS DURING THE FIRST HOURS OF LIFE

I *After Normal and Short Gestation and Gestation Complicated by Hypertension With Special Reference to the Small for Dates Syndrome*

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Many babies who at birth are unduly small for the period of gestation (small for dates = SFD) show certain clinical features indicating impairment of prenatal nutrition (36)

The homeostasis of plasma free amino acids changes characteristically in experimental protein undernutrition (low intake of dietary nitrogen) (1 2 28 31) This change is also seen in the clinical syndrome of protein deficiency (3 11 16 27 33 35)

The aim of the present study is to investigate whether this characteristic change in the homeostasis of plasma free amino acids is also valid for the SFD newborn The SFD syndrome has a multifactorial background (5) This study deals with SFD babies of mothers with hypertensive disorder of pregnancy This maternal condition has been shown to be associated with low birth weight and short length for gestational age of the babies in an earlier investigation (20)

MATERIAL

The material is based on a study of 47 newborns (18 normals 9 early borns and 20 newborns of mothers with hypertension during pregnancy) Essential neutral amino acid levels of plasma were determined in all cases at different times during the first hours of life In 25 of the cases (9 normals, 8 early borns and 8 newborns of mothers with hypertension during pregnancy) one complete determination of 18 free amino acid levels was made

The mothers were all without history of previous disease The normal and short gestation group had a blood pressure below 140/90 and no protein or glucose in their urine during the actual pregnancy All of them were Rh positive and no child showed hyperbilirubinaemia during the experimental period The normals and early borns had a crown vaginal delivery while 6 of the hypertensive mothers were delivered by caesarean section because of low urinary oestriol excretion in 5 cases and intrauterine asphyxia in one case The cord was wound 3 times around the neck of the asphyxial child and as cord obstruction is known to lower the amino acid levels of cord plasma (8) this case is separately presented in Fig 1 and was excluded from the survey The criteria of hypertensive disorder of pregnancy have been given in an earlier work (20) There was no correlation between time of noted onset of hypertension and birth weight reduction The mothers were treated from the time of first noted hypertension with Apresolin® and Renese® (Polythiazide) with extra peroral potassium All three of the newborns of the hypertensive group whose mothers had not been treated during pregnancy had a birth weight or length below −2 S D according to the Swedish standard for gestational age and sex (12) Oestriol excretion below 10 mg/day was noted during the last week of pregnancy in 5 hypertensive cases out of whom one had a birth weight or length below −2 S D

The Apgar score was normal at 4 min of age and through the experimental period The normals and early borns had a birth weight and length within ±1 S D for gestational age and sex The newborns of

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radiological size of the kidney suggests various etiologies for the parenchymal reduction

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REFERENCES

1 Bernstein L M & Hamby W M Unilateral urine sampling utilizing external ureteral compression *New Eng J Med 268* 1093 1963
2 Bernstein J Developmental abnormalities of the renal parenchyma—renal hypoplasia and dysplasia *Path Ann 3* 213 1968
3 Coelho J B & Bradley S E Function of the *nephron population during haemorrhagic hypotension* in the dog with special reference to the effects of osmotic diuresis *J Clin Invest 43* 386 1964
4 Cox C E & Hinman F Jr Experiments with induced bacteriuri vesical emptying and bacterial growth on the mechanism of bladder defence to infection *J Urol 86* 739 1961
5 Cox C E Lacy S S & Hinman F Jr Urethra and its relationship to urinary tract infection II Urethral flora of female with recurrent urinary infection *J Urol* 99 632 1968
6 Emmett J L Alvarez Ierena J J & McDonald J R Atrophic pyelonephritis versus congenital renal hypoplasia *JAMA 148* 1970 1952
7 Ericsson N O & Ivemark B I Renal dysplasia and pyelonephritis in infants and children Part I *Arch Path 66* 255 1958
8 Ericsson N O & Ivemark B I Renal dysplasia and pyelonephritis in infants and children Part II *Arch Path 66* 264 1958
9 Hamby W M & Bernstein L M Measurement of unilateral renal function by external ureteral compression *Circ 31* 679 1965
10 Hamby W M Blumberg M B Simpson K & Bernstein L M Unilateral renal function measurement external ureteral compression compared with ureteral catheterization *J Lab Clin Med 71 722 1968*
11 Heyrowsky A A new method for the determination of inulin in plasma and urine *Clin Chim Acta 1* 470 1956
12 Hodson C J The radiological contribution toward the diagnosis of chronic pyelonephritis *Radiology 88* 857 1967
13 Ladefoged J & Pedersen F Relationship between roentgenological size of the kidney and the kidney function *J Urol 99* 239 1968
14 Marshall A G The persistence of foetal structure in pyelonephritic kidneys *Brit J Surg 41* 38 1953
15 — Scars of the renal cortex *J Path Bact 71* 95 1956
16 — Scars of the renal cortex *J Path Bact 95* 225 1968
17 Michie A J & Michie C R Kidney function in unilateral pyelonephritis I Clinical data *Amer J Med 22* 179 1957
18 Michie A J Michie C R & Ratnu M C Kidney function in unilateral pyelonephritis II Physiologic interpretations *Amer J Med 22* 190 1957
19 *Norden C W Green G M & Kass E H* Antibacterial mechanisms of the urinary bladder *J Clin Invest 47* 2689 1968
20 Smith H W *The kidney Structure and function in health and disease* Oxford University Press New York 1951
21 Stanley T A Fair W R Timothy M M & Chung H K Antibacterial nature of prostatic fluid *Nature 218* 444 1968
22 Strauss M B & Welt L G *Diseases of the kidney* Little Brown and Comp Boston 1963 p 469
23 *Templeton A W & Thompson I M* Aortographic differentiation of congenital and acquired small kidneys *Arch Surg 97 114 1968*
24 Winberg J Renal concentration capacity during acute nonobstructive urinary tract infections in infancy and early childhood *Acta Paediat Scand 47* 635 1958
25 — Renal function studies in infants and children with acute nonobstructive urinary tract infections *Acta Paediat Scand 48 577 1959*
26 Vivaldi E Cotran R Zangwill D P & Kass E H Ascending infection as a mechanism in the pathogenesis of experimental non obstructive pyelonephritis *Proc Soc Exp Biol Med 102* 242 1959

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Key words Childhood pyelonephritis renal parenchymal reduction renal dysplasia split function test

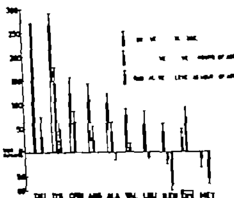


Fig 3 Change of the free amino acid levels of plasma during the first hours of life of the fasting normal newborn The 4 hour level is indicated if different from the cord vein level (19) the 48 hour level if different from the 4 hour level The other 8 amino acid levels investigated showed a not significant decrease and urea a not significant increase during this period The normal infant level is that of infants 9 months to 2 years of age (29)

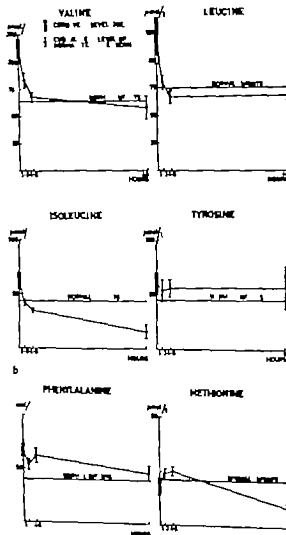


Fig 4 Mean±2 s e of the plasma levels of neutral essential amino acids of the normal newborn during the first hours of life The horizontal line indicates the normal fasting levels of infants 9 months to 2 years of age (29)

β hydroxybutyrate was determined according to Persson (24) Plasma albumin was determined by electrophoresis on cellulose acetate (30) Blood glucose was estimated by the enzymatic method of Marks (22)

RESULTS

Normal newborns At 1–3 hours of age the venous plasma levels of the branch-chained amino acids (valine leucine isoleucine) and phenylalanine showed a decrease from cord levels (Fig 4)

At 4 hours of age the branch-chained amino acid levels showed a further decrease The levels of lysine and alanine were considerably lower than their cord levels while the glycine level was increased The ornithine/arginine quotient seems to be increased compared to cord value (Fig 3) Urea was not significantly increased (Fig 6 *a*) See Table 1

Table 1 *Normal newborns*

Cord vein plasma levels of free amino acids CB(19) are compared to those of the cubital vein plasma at 4 hours of age CUB Mean and s D are given in μmol/l plasma

Amino acid			Mean	s D	Significance of difference
Valine	CB	10	224	25	
	CUB	12	139	20	
Leucine	CB	10	118	32	
	CUB	12	67	13	
Isoleucine	CB	10	62	12	
	CUB	12	36	5	
Lysine	CB	10	318	32	
	CUB	5	212	33	
Alanine	CB	10	441	73	
	CUB	9	311	65	
Glycine	CB	10	239	35	
	CUB	9	293	48	

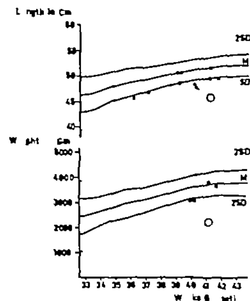


Fig 1 Weight and length at birth in relation to the length of the gestational period of the cases where pregnancy was complicated by hypertension The filled circles represent the group characterized as the growth retarded group The crosses represent those growth retarded newborns who developed neonatal hypoglucosaemia The encircled filled circles represent the case with intrauterine asphyxia For the sake of simplicity both sexes have been plotted onto the Swedish standard for boys (12)

mothers with hypertension had weight and length below −1 SD in 12/20 cases These babies are referred to as the growth retarded group (Fig 1) weight or length below −2 SD was present in 10/20 cases and weight and length below −2 SD in 6/20 cases The normals had a gestational age of 38–41 weeks the early born less than 36 weeks and the hypertensive group 36–41 weeks The babies showed no malformations or infections during the neonatal period

EXPERIMENTAL

The babies were all transferred to an incubator in a unit for newborns within one hour after delivery *The stomach was emptied by tubing and sucking im*mediately after birth

1–2 ml of blood was drawn from the cubital vein into a heparinized tube at different times during the first hours of life The first feed was given at 6 hours of age In 12 cases where the first feed (10 g of pooled breastmilk) was given at 4 hours of age a sample of 3–4 ml of blood was drawn before and one hour after the feed In these cases blood for glucose estimation was collected by heel puncture before the first feed After this the full term babies were taken out of the incubator Subsequent samples were drawn 3–4 hours after the latest feed (immediately before the next feed) In 6 normals the first period of fasting was prolonged and a blood sample of 3–4 ml was drawn before and one hour after the first feed (30 g of pooled breastmilk) at 44–51 hours of age These babies had received 10–20 ml of 10% glucose solution 6 times daily during this period Urine was collected in a sterile plastic uri bag at 4–7 hours of age from 16 cases (all boys because of the greater possibility of preventing contamination) These urine samples were frozen immediately after urination

ANALYTICAL METHODS

Collection of samples storage and deproteinization has been described earlier (19) The levels of 18 free amino acids and urea were determined in the 4 and 48 hour samples of 0 5–1 0 ml plasma with a Beckman 120 B automatic amino acid analyzer (19)

The neutral essential amino acid levels were determined in 0 3 ml of plasma according to the ion exchange chromatography method described in an *earlier report (21)*

A further modification allowed reproducible determinations of proline glycine alanine and valine levels of the urine samples The pH of the urine was brought to 2 0 by addition of 6 *N* HCl Without further preparation 0 2–0 5 ml were put directly onto the column The pH of the elution buffer was lowered from 3 43 to 3 06±0 03 by addition of concentrated HCl The temperature was 60 2 The two fold linear amplification of the recorder was omitted (Fig 2)

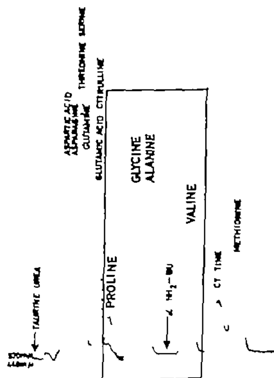


Fig 2 Modified standard run of ion-exchange chromatography for determination of urinary concentrations of proline glycine alanine and valine 50 mμmol of each amino acid was loaded on the column

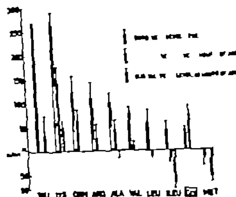


Fig 3 Change of the free amino acid levels of plasma during the first hours of life of the fasting normal newborn The 4 hour level is indicated if different from the cord vein level (19) the 48 hour level if different from the 4 hour level The other 8 amino acid levels investigated showed a not significant decrease and urea a not significant increase during this period The normal infant level is that of infants 9 months to 2 years of age (29)

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	CUB	12	139	20	
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	CUB	12	67	13	
Isoleucine	CB	10	64	12	
	CUB	12	36	5	
Lysine	CB	10	318	32	
	CUB	5	212	33	
Alanine	CB	10	441	75	
	CUB	9	311	65	
Glycine	CB	10	239	33	
	CUB	9	293	48	

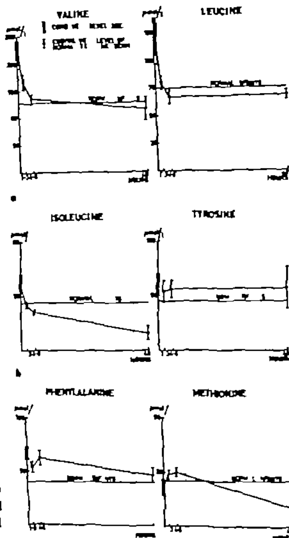


Fig 4 Mean ± 2 S E of the plasma levels of neutral essential amino acids of the normal newborn during the first hours of life The horizontal line indicates the normal fasting levels of infants 9 months to 2 years of age (29)

phenylalanine showed a decrease from cord levels (Fig 4)

At 4 hours of age the branch-chained amino acid levels showed a further decrease The levels of lysine and alanine were considerably lower than their cord levels while the glycine level was increased The ornithine/arginine quotient seems to be increased compared to cord value (Fig 3) Urea was not significantly increased (Fig 6 a) See Table 1

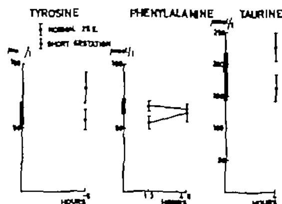


Fig 5 Change of the free amino acid levels of plasma of the early born during the first hours of life Mean ± 2 s.e. is indicated

When fasting was prolonged until 48 hours of age isoleucine, lysine and alanine levels showed a further decrease The taurine and methionine levels were low compared to cord levels (Fig 3)

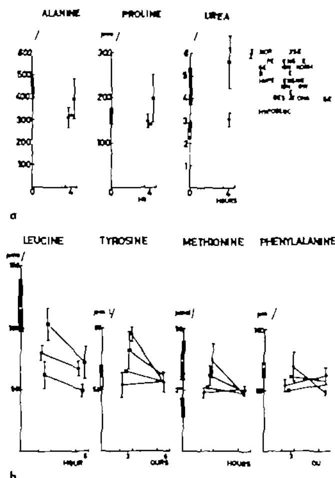


Fig 6 Differences from the normal free amino acid levels of plasma in the newborns of mothers with hypertensive disorder

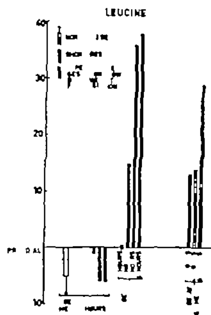


Fig 7 Plasma leucine level one hour after the first feed of 10 g of pooled breastmilk during the first day of life and 30 g after prolonged neonatal starvation

One hour postprandial levels after 10 g of breastmilk at 4 hours of age showed no significant or consistent differences from preprandial levels Thirty grams of breastmilk after 48 hours of fasting gave an increase of leucine isoleucine and a decrease of glycine and the glycine/valine quotient of plasma one hour postprandially (Fig 7)

Newborns with short gestational period At 1–3 hours of age there were higher levels than in normals of phenylalanine and after 4–6 hours of fasting higher levels than in normals of taurine and tyrosine (Fig 5) One hour after 10 g of breastmilk, given at 4 hours of age there seemed to be a consistent non significant increase of alanine only

Newborns of mothers with hypertensive disorder Differences from the normal and short gestational group

After 1–3 and 4–6 hours of neonatal fasting the branch-chained amino acids showed lower levels if pregnancy had been complicated by hypertension In the case of low birth weight for gestational age however the levels were increased at 1–3 hours and normal at 4–6 hours There seems to be a more rapid decrease of the

Table 2 *Newborns of mothers with hypertensive disorder and normal weight at birth according to gestational age (A) and newborns of mothers with hypertensive disorder and low weight at birth according to gestational age (B)*

The free amino acid levels of cubital vein plasma are compared to normal levels at the same age Mean and s d are given in μmol/l plasma

		n	Mean	s d	Significance of difference
1 3 hours of age					
Leucine	A	4	62	11	
	Normal	10	80	9	
	B	10	103	21	
4-6 hours of age					
Leucine	A	6	49	6	
	Normal	18	67	12	

branch-chained amino acid levels of plasma from 1-3 to 4-6 hours of age (Fig 6 *b*) In the growth retarded group the same tendency towards increased levels at 1-3 hours of age and an increased disappearance from plasma thereafter was also seen in the other essential amino acid levels studied See Table 2

At 4 hours of age the urea plasma level was higher than normal when pregnancy had been complicated by hypertension Alanine and proline showed a tendency towards increased levels in the growth retarded group In one growth retarded case where a marked neonatal hypoglucosaemia with jitteriness was observed (8 mg at 12 hours of age) the increase of the alanine and proline levels of plasma was marked (Fig 6 *a*)

One hour after 10 g of breastmilk given at 4 hours of age the levels of leucine proline histidine and phenylalanine were increased in the growth retarded group (Fig 7)

The urine collected at 4-7 hours of age showed no significant differences between the ratios of the concentrations of the different amino acids compared to the normal material There was a tendency towards low urinary creatinine and polyuria in the hypertensive group

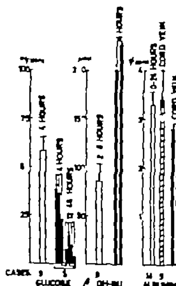


Fig 8 Glucose and β hydroxybutyrate levels at 4 hours of age and cord vein albumin level Unfilled columns represent normal mean+2 s e. Filled columns represent individual levels of these investigated newborns of mothers with hypertensive disorder during pregnancy who had low birth weight for gestational age The striped column represents cord vein plasma level+range of those newborns of mothers with hypertensive disorder of pregnancy who had normal birth weight for gestational age The normal albumin level during the first day of life (mean+2 s e.) is according to Saito *et al* (25)

Low blood glucose levels and a tendency towards higher levels of β hydroxybutyrate at 4 hours of age and lower plasma albumin level in cord blood of the growth retarded *group* is demonstrated in Fig 8

DISCUSSION

The only changes of the plasma free amino acid levels found during the initial fast of the *normal newborn* (Fig 3) are identical to those found in calorie deficiency Experimental calorie undernutrition leads to a characteristic decline of the branch-chained amino acid and alanine levels of plasma while the glycine level and the ornithine/arginine quotient increase (1 13) The lysine level decreases in experimental protein restriction in addition to the above mentioned changes of amino acid levels while the alanine level increases (1 2 28 31)

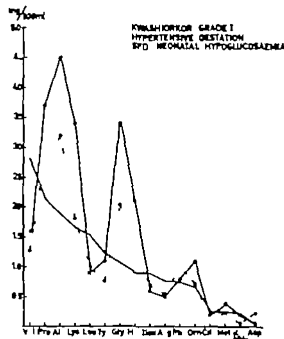


Fig 9 The plasma aminogram of Kwashiorkor Grade I (16) and of a newborn of mother with hyper tensive disorder during pregnancy who had low birth weight and short length for gestational age and de veloped neonatal hypoglucosaemia Threonine serine and tryptophane levels were not determined (19) Taurine is excluded as its foetal metabolism seems to be unique to the foetus (21) Normal levels of infants (16) are indicated by the declining solid line

These changes in the homeostasis of free amino acids of plasma are evident less than 48 hours after the reduction of protein intake in normal infants (28)

The findings are in agreement with the de monstrated early postnatal intensification of hepatic accumulation of amino acids in the guinea pig (7) and the increased mobilization and utilization of fat during the neonatal period (23 32) The fast urinary excretion and continuous drop of the plasma level of taurine has been discussed earlier (21) Equivalence of essential amino acid concentration in portal and radial venous blood has been demon strated (10) *Time study of cubital venous* plasma in individual cases of the present inves-tigation, from within half an hour after birth shows a continuous drop of the essential amino acid levels studied to the levels at 4 hours of age This justifies a comparison of cord vein and 4-hour-of age cubital vein levels

In the *newborn of mothers with hypertensive disorder* the resistance to decline" of the ala nine and proline levels, and the faster decrease of the essential amino acid levels seem to be correlated to the severity of the SFD syndrome (Fig 6) These changes are the same as those found in protein deficiency of children (3 11 16 27 33 35) The alanine level that de creases in experimental calorie undernutrition and increases in experimental protein under nutrition also increases in the protein defi ciency of Kwashiorkor Grade I (16) Splanch nic uptake of alanine greatly exceeds that of all other amino acids (6) Proline is also increased in the protein deficiency of kwashiorkor and in experiments with low and excess protein intake in children and infants (28) The increased le vels of proline seem to be characteristic of pro-tein malnutrition of children independent of the type of malnutrition

Apart from a more pronounced change in the hypoglycosaemic growth retarded newborn (Fig 9) there also seems to be a resistance *to decline of the tyrosine level This can be* explained by the fact that these children are born slightly early the known deficiency of *p* hydroxy phenylpyruvate oxidase activity in the liver of foetuses (17) and the high levels of tyrosine found in the plasma of early borns at 4–6 hours of age (Fig 5) as well as in some babies later during the neonatal period (4 18)

Thus the growth retarded newborns of mothers with hypertensive disorder of preg nancy showed the signs that have been called the earliest biochemical abnormality of mal nutrition (34) The aminogram is compatible with protein deficiency

In the growth retarded group the first breastmilk *feed of 10 g at 4 hours of age* causes a one hour postprandial increase of above all the leucine level of plasma This change is equalled in the normal fasting new born by 30 g at 48 hours of age (Fig 7) In the normal full term newborn the amino acid levels have been reported to be unchanged one hour postprandially (15) The leucine content of breastmilk protein is among the highest for

any amino acid (26) and the branch-chained amino acids are characterized by a fast intestinal absorbance (14). The general tendency was towards a postprandial increase of the amino acid levels with fastest neonatal decline and decrease of the amino acid levels that increase during the initial fast. This means that the first feed of the growth retarded newborn is accompanied by a greater penprandial fluctuation of the free amino acid levels of plasma especially if the first feed is delayed. This might be of clinical importance as high protein intake seems to be able to aggravate the severity of the hypoglycaemic attacks occasionally seen in growth retarded newborns (9).

SUMMARY

During the first hours of extrauterine life the venous plasma of the fasting normal newborn shows a fast decline of valine, leucine, isoleucine, lysine and alanine levels while the glycine level and the ornithine/arginine quotient seem to increase. These changes are not caused by urinary excretion and are the same as those found in calorie undernutrition.

The fasting newborn of mothers with hypertension of pregnancy who are underweight and short for gestational age have increased levels of valine, leucine and isoleucine during the first hours after birth and a tendency towards a more rapid decline of the levels thereafter. The alanine and proline levels seem to resist the general decline during the initial fast. These changes in the homeostasis of plasma free amino acids are not due to short gestational period. They are the same as those found in protein deficiency.

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REFERENCES

1 Adibi S A. Influence of dietary deprivations on plasma concentration of free amino acids of man. J Appl Physiol 25: 52, 1968.

2 Arroyave G. The estimation of relative nutrient intake and nutritional status by biochemical methods: Protein. Am J Clin Nutr 11: 447, 1962.

3 Arroyave G, Wilson D, de Funes C & Behar M. The free amino acids in blood plasma of children with kwashiorkor and marasmus. Am J Clin Nutr 11: 517, 1962.

4 Bloxam H R, Day M G, Gibbes N K & Woolf L I. An inborn defect in the metabolism of tyrosine in infants on a normal diet. Biochem J 77: 320, 1960.

5 Butler N R & Bonham D G. Perinatal mortality. Livingstone, Edinburgh 1963.

6 Carlsten A, Hallgren B, Jagenburg R, Svanborg A & Werkö L. Arteriohepatic venous differences of free fatty acids and amino acids. Acta Med Scand 181: 199, 1967.

7 Christensen H N & Clifford J B. Early postnatal intensification of hepatic accumulation of amino acids. J Biol Chem 238: 1743, 1963.

8 Clemetson C A B & Churchman J. The placental transfer of amino acids in normal and toxaemic pregnancy. J Obstet Gynec Brit Emp 61: 364, 1954.

9 Cochrane W A. Studies in the relationship of amino acids to infantile hypoglycemia. Am J Dis Child 99: 476, 1960.

10 Denton A E & Elvehjem C A. Availability of amino acids in vivo. J Biol Chem 206: 449, 1954.

11 Edozien J C, Phillips E J & Collis W R F. The free amino acids of plasma and urine in kwashiorkor. Lancet I: 615, 1960.

12 Engström L & Sterky G. Standardkurvor för vikt och längd hos nyfödda barn. Läkartidningen 51: 4922, 1966.

13 Felig P, Owen O E, Wahren J & Cahill G F Jr. Amino acid metabolism during prolonged starvation. J Clin Invest 48: 584, 1969.

14 Frame E G. The levels of individual free amino acids in the plasma of normal man at various intervals after a high protein meal. J Clin Invest 37: 1710, 1958.

15 Ghadimi H & Pecora P. Plasma amino acids after birth. Pediatrics 34: 182, 1964.

16 Holt L E, Snyderman S E, Norton P M, Roitman E & Finch J. The plasma aminogram in kwashiorkor. Lancet II: 1343, 1963.

17 Kretchmer N. Enzymatic pattern during development: an approach to a biochemical definition of immaturity. Pediatrics 23: 606, 1959.

18 Levine S Z, Marples E & Gordon H H. A defect in the metabolism of tyrosine and phenylalanine in premature infants. J Clin Invest 20: 199, 1941.

19 Lindblad B S & Baldesten A. The normal venous plasma free amino acid levels of nonpregnant women and of mother and child during delivery. Acta Paediat Scand 56: 37, 1967.

20 Lindblad B S & Zetterström R. The venous plasma free amino acid levels of mother and child during delivery. II. After short gestation and gestation complicated by hypertension with

special reference to the small for dates syndrome *Acta Paediat Scand 57* 195 1968
21 Lindblad B S & Baldesten A Time studies on free amino acid levels of venous plasma during the neonatal period *Acta Paediat Scand 58* 252 1969
22 Marks V An improved glucose oxidase method for determining blood CSF and urine glucose *Clin Chim Acta 4* 395 1959
23 Persson B & Gentz J The pattern of blood lipids glycerol and ketone bodies during the neonatal period infancy and childhood *Acta Paediat Scand 55* 353 1966
24 Persson B Determination of plasma acetoacetate and D β hydroxybutyrate in newborn infants by an enzymatic fluorimetric micromethod To be published *Scand J Clin Lab Invest* 1969
25 Saito M Gittleman I F Pincus J B & Sobel A E Plasma protein patterns in premature infants of varying weights on the first day of life *Pediatrics 17* 657 1956
26 Saito & Furuichi *Studies on human milk* Research laboratory Snow Brand Milk Products Co 3-21 Akabanchita Kitaku Tokyo 1965
27 Saunders S J Truswell A S Barbezat G O Wittman W & Hansen J D L Plasma free amino acid pattern in protein calorie malnutrition Reappraisal of its diagnostic value *Lancet II* 795 1967
28 Snyderman S E Holt L E Jr Norton P M Roitman E & Phansalkar S V The plasma aminogram I Influence of the level of protein intake and a comparison of whole protein and amino acid diets *Ped Res 2* 131 1968
29 Soupart P Free amino acids of blood and urine in the human *In* J T Holden (ed) *Amino acid pools* Elsevier Press Inc New York 1962
30 Spinco Division *Electrophoresis on cellulose acetate membranes in the microzone system* Beckman Instruments Inc Palo Alto California 1969
31 Swendseid M E Griffith W H & Tuttle S G The effect of a low protein diet on the ratio of essential to nonessential amino acids in blood plasma *Metabolism 12* 96 1963
32 Van Duyne C Parker H & Holm L Metabolism of free fatty acids during perinatal life of lambs *Am J Obstet 91* 277 1965
33 Westall R G Roitman E de la Pena C Rasmussen H Cravioto J Gomez F & Holt L E Jr The plasma amino acids in malnutrition *Arch Dis Child 33* 499 1958
34 Whitehead R G Rapid determination of some plasma amino acids in subclinical kwashiorkor *Lancet I* 250 1964
35 Whitehead R G & Dean R F A Serum amino acids in kwashiorkor I Relationship to clinical condition *Am J Clin Nutr 14* 313 1964
36 Wigglesworth J S Foetal growth retardation *Brit Med Bull 22* 13 1966

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THE VENOUS PLASMA FREE AMINO ACID LEVELS DURING THE FIRST HOURS OF LIFE

II *In a Lower Socio economic Group of a Refugee Area in Karachi West Pakistan with Special Reference to the Small for Dates Syndrome*

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The marked differences in birth weight between different races seems to be largely dependent on socio-economical differences (10) Since protein undernutrition is common in many economically developing countries the question arises if protein undernutrition is of significance to the lower birth weight seen in these areas (9 19) Protein undernutrition of the pregnant rat and dog leads to growth retardation and late neurological sequelae of the offspring (5 12 18 21)

Certain changes in the homeostasis of plasma free amino acids are characteristic of protein deficiency in the human (3 6 17 20 25 26) The aim of the present investigation is to find out whether the babies of a low socio economical group of West Pakistani women show these signs of protein deficiency The free amino acid levels of cord vein plasma of this socio-economical group have been reported earlier (16) The plasma levels of free amino acids during the immediate postnatal period of Swedish normal early born and growth retarded newborns have been given in a previous paper (17)

This work was made possible through grants from AB Semper Fund for Nutritional Research and grant no 2383 from the Swedish Medical Research Council

MATERIAL

The group investigated is the low socio-economical group more extensively described earlier (16) The mothers were nutritionally characterized by daily low consumption of vegetable protein during both the prematernal as well as maternal stage Their venous plasma at the time of delivery had the increased glycine level and glycine/valine quotient characteristic of protein deficiency Among the infants of this group there was a greater frequency of low birth weight and short length for gestational age than in a middle-class group with the same racial and cultural background when the birth weights and lengths of both groups were compared to an "ideal" standard (7) Length of pregnancy given during consultation with the mother was 38–41 weeks The newborn showed no neurological signs or feeding difficulties typical of short pregnancy upon investigation during the 3-day postnatal care There was no hypertension of the mothers at the time of delivery and no signs of intra or extrauterine asphyxia

METHODS

The experimental conditions were identical to those of the normal material (17) except that during the experimental period the babies in the present investigation were kept covered with a quilt in bed and not in an incubator

Collection of samples storage and deproteinization has been described earlier (14) In 7 cases a complete analysis of all neutral and acidic amino acid and urea levels at 4 hours of age was made on 0 5–1 0 ml plasma with a Beckman 120 B automatic amino acid analyzer (14) In all 21 cases neutral essential amino acids were determined in samples taken at different hours during the first 6 hours of life 0 3 ml of plasma

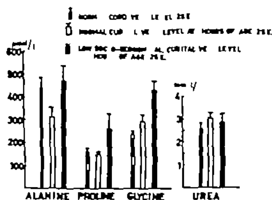


Fig 1 Differences from the normal free amino acid levels of plasma at 4 hours of age

was used according to the method described in an earlier report (15)

Plasma protein levels were determined by electrophoresis on cellulose acetate (23)

RESULTS

At 4 hours of age there was a significant increase of the alanine, proline glycine and taurine levels compared to the normal levels at this age (Fig 1) The branch chained amino acid levels of plasma showed a delay in their normal decline (Fig 2) The other amino acids investigated showed normal plasma levels

Cord vein plasma concentrations of protein components were normal

DISCUSSION

The increased plasma levels of alanine proline and glycine found are characteristic of

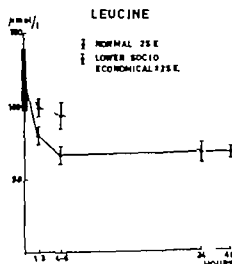


Fig 2 Leucine plasma levels during the immediate neonatal period

Table 1 *Free amino acid levels of cubital vein plasma compared to normal levels at the same age (17)*

Mean and S D are given in µmol/l plasma * = significant 0 1 < p ≤ 1 ** = highly significant p < 0 1

		n	Mean	S D	Significance of difference
1–3 hours of age					
Leucine		20	99	14	*
	Normal	10	80	9	
4–6 hours of age					
Leucine		21	94	20	* *
	Normal	18	67	12	
4 hours of age					
Glycine		7	428	57	**
	Normal	9	293	48	
Alanine		7	469	95	**
	Normal	9	311	65	
Proline		7	261	93	**
	Normal	9	147	24	
Cord vein levels (16)					
Glycine		9	320	57	
	Normal (14)	10	239	32	
Proline		9	210	36	**
	Normal	10	160	28	

protein deficiency in infants and children, both after experimental protein restriction (1 2 22 24) and in the clinical syndrome of Kwashiorkor Grade I (11)

A temporary increase of the branch chained amino acid levels of plasma with a rapid fall thereafter is reported to occur in experimental starvation of adults (1 8) and was found in newborn small for dates (undue low birth weight for gestational age) of mothers with hypertensive disorder of pregnancy (17) Normal urea tyrosine and phenylalanine levels contradict the possibility of an increased peripheral release of amino acids in the newborns (4) The findings are not compatible with short pregnancy (13 17)

The taurine level was increased at 4 hours of age compared to the normal at this age Increased taurine level has been reported in

Kwashiorkor Grade I (22) As the normal foetal metabolism of taurine seems to be unique to the foetus (15) no conclusions can be drawn from this finding

The increased levels of glycine and the glycine/valine quotient, typical of the calorie deficiency of the first fasting period of the normal newborn (17) and of proline characteristic of protein malnutrition of infants (22) were seen already in cord vein plasma of this socio-economical group (16) See Table 1 The alanine level normally declines in plasma rapidly after birth (17) The resistance to decline of alanine found in this group is not compatible with calorie deficiency (1 8) The findings suggest a protein deficiency starting during intrauterine life

SUMMARY

The plasma aminogram was investigated in newborns from a low socio-economical group of West Pakistan with an increased frequency of the small for dates syndrome

The plasma levels of proline and glycine increased during the first hours of life to levels significantly higher than those of a Swedish normal material The alanine levels showed a resistance to decline during this period The branch-chained amino acid levels of plasma showed a fast decline after a temporary resistance to decline

The plasma free amino acid levels changed during the first hours of life to a pattern which is the same as that seen in protein deficiency

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REFERENCES

1 Adibi S A Influence of dietary deprivations on plasma concentration of free amino acids of man *J Appl Physiol 25* 52 1968

2 Arroyave G The estimation of relative nutrient intake and nutritional status by biochemical methods Protein *Am J Clin Nutr 11* 447 1962

3 Arroyave G Wilson D de Funes C & Behar M The free amino acids in blood plasma of children with kwashiorkor and marasmus *Am J Clin Nutr 11* 517 1962

4 Bremer H J & Neumann W Phenylalanine toleranz bei Fruhgeborenen reifen Neugeborenen Sauglingen und Erwachsenen *Klin Wschr 44* 1076 1966

5 Chow B F & Lee C J Effect of dietary restriction of pregnant rats on body weight gain of the offspring *J Nutr 82* 10 1964

6 Edozien, J C Phillips E J & Collis, W R F The free amino acids of plasma and urine in kwashiorkor *Lancet I* 615 1960

7 Engstrom L & Sterky G Standardkurvor for vikt och langd hos nyfodda barn *Lakartidningen 31* 49.. 1966

8 Fehg P Owen O E Wahren J & Cahill G F Jr Amino acid metabolism during prolonged starvation *J Clin Invest 48* 584 1969

9 Ghosh S & Daga, S Comparison of gestational age and weight as standards of prematurity *J Pediat 71* 173 1967

10 Groenwald P Growth pattern of the normal and the deprived fetus *In* J H P Jonxis H K A Visser & J A Troelstra (eds) *Aspects of prematurity and dysmaturity* Nutricia Symposium H E Stenfert Kroese N V Leiden 1968

11 Holt L E Snyderman S E Norton P M Roitman E & Finch J The plasma aminogram in kwashiorkor *Lancet II* 1343 1963

1. Hsueh A M Agustin C E & Chow B F Growth of young rats after differential manipulation of maternal diet *J Nutr 91* 195 1967

13 Kretchmer N Enzymatic patterns during development an approach to a biochemical definition of immaturity *Pediatrics 23* 606 1959

14 Lindblad B S & Baldesten A The normal venous plasma free amino acid levels of non pregnant women and of mother and child during delivery *Acta Paediat Scand 56* 37 1967

15 Lindblad B S & Baldesten A Time studies on free amino acid levels of venous plasma during the neonatal period *Acta Paediat Scand 58* 252 1969

16 Lindblad B S Rahimtoola R J Said M Haque Q & Khan N The venous plasma free amino acid levels of mother and child during delivery III In a lower socio-economic group of a refugee area in Karachi West Pakistan with special reference to the small for dates syndrome *Acta Paediat Scand 58* 497 1969

17 Lindblad B S The venous plasma free amino acid levels during the first hours of life After normal and short gestation and gestation complicated by hypertension. With special reference to the small for dates syndrome *Acta Paediat Scand 59* XXX 1970

18 Platt B S Experimental protein calorie deficiency *in* R A McCance & Elsie M Widdowson (eds) *Calorie deficiencies and protein deficiencies* J A Churchill Ltd London 1968
19 Rahimtoola R J Mir S & Baloch S Low birth weight the small for dates syndrome and perinatal mortality in a low family income group *Acta Paediat Scand 57* 534 1968
20 Saunders S J Truswell A S Barbezat G O Wittman W & Hansen J D L Plasma free amino acid pattern in protein-calorie malnutrition Reappraisal of its diagnostic value *Lancet II 795 1967*
21 Simonson M Yu W Anilane J K Sherwin R & Chow B F Studies of development in progeny of underfed mother rats *Fed Proc 26* 519 1967
22 Snyderman S E Holt L E Jr Norton P M Roitman E & Phansalkar S V The plasma aminogram I Influence of the level of protein intake and a comparison of whole protein and amino acid diets *Ped Res 2* 131 1968
23 Spinco Division *Electrophoresis on cellulose acetate membranes in the micro one system* Beckman Instruments Inc Palo Alto California 1969
24 Swendseid M E Griffith W H & Tuttle S G The effect of a low protein diet on the ratio of essential to nonessential amino acids in blood plasma *Metabolism 12* 96 1963
25 Westall R G Roitman E de la Pena C Rasmussen H Cravioto J Gomez F & Holt L E Jr The plasma amino acids in malnutrition *Arch Dis Child 33* 499 1958
26 Whitehead R G & Dean R F A Serum amino acids in kwashiorkor I Relationship to clinical condition *Am J Clin Nutr 14* 313 1964

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C REACTIVE PROTEIN IN SERUM FROM INFANTS AS DETERMINED WITH IMMUNODIFFUSION TECHNIQUES

I *Healthy infants*

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Earlier clinical studies of C reactive protein (CRP) in serum have usually been performed with the capillary tube precipitation method (1) The results obtained with this method vary considerably due to differences in the antisera (4) Investigations using immunodiffusion techniques have shown a greater sensitivity and accuracy of the determination of CRP (5 7) Sera from infants have not yet been studied with these methods A test which can be used as an indicator of infection is of special interest in this age group since here the usual clinical and laboratory criteria for infection are often less reliable—especially in the young infants Felix *et al* (3) reported that with the capillary tube precipitation technique one third of their infected infants had no demonstrable CRP in their sera It should be of interest to see whether or not the more sensitive immunodiffusion methods could detect CRP in more of these infected cases and if the CRP concentration in serum corresponds to the clinical condition As a basis for such an evaluation of the possible clinical usefulness of these methods for the detection of CRP in this age a group of normal infants was studied

MATERIAL AND METHODS

Capillary blood samples were collected from 100 infants from 1 15 months of age on their regular health controls at the child welfare clinic Any pathological findings on the clinical examination were recorded Infants with demonstrable CRP were taken back for repeated blood sampling and renewed clinical examination

The comparative double diffusion in gel technique was used for the detection of CRP (5 7) The sensitivity limit of this technique is about 0 5 µg/ml Positive samples were quantitated with the single radial immunodiffusion technique as earlier described (5) This method detects concentrations exceeding 1 µg/ml Concentrations between 0 5 and 1 µg CRP/ml are recorded as trace amounts Commercially available anti-CRP from sheep and goat (Hyland Lab Los Angeles California) was used

RESULTS

The age distribution of the infants is illustrated in Fig 1 where the CRP containing sera are also indicated There is no evident relation between age and the frequency of CRP containing samples as judged from these small groups

The presence of CRP correlated to the clinical findings is recorded in Table 1 Ninety eight of the 100 infants were healthy on clinical examination In seventeen of these apparently healthy infants CRP was found although in thirteen of the sera it was merely present in trace amounts detectable only with the double diffusion method and not with the quantitative radial diffusion technique Four of these infants had suffered from upper respiratory tract infections within a week before

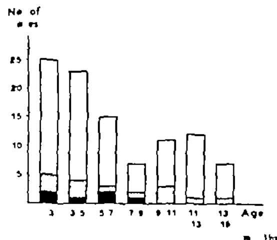


Fig 1 Number of sera containing CRP related to age □ CRP not demonstrable /// trace amounts of CRP ■ CRP > 1 μg/ml

the sampling and recovered with no detectable CRP on reexamination The history was otherwise uneventful in these thirteen infants as well as in the four cases where CRP was quantifiable (11 6 3 and 2 μg/ml) The infant with the highest amount of CRP that had been apparently healthy upon examination was two weeks later found to have pyelonephritis and anatomical abnormalities of the kidneys At that time it still had CRP in its serum The three other of the four with CRP concentrations in serum exceeding 1 μg/ml had no demonstrable CRP upon check-up

The two of the 100 infants which had positive findings on clinical examination (bronchitis) both had CRP in their sera one with trace amounts and one with 16 μg/ml

Table 1 *Relation between the presence or absence of CRP and the result of clinical examination*

CRP		Clinical examination Positive	Negative	Total
Present	> 1 μg/ml	1	4	5
	trace amounts (0 5–1 μg/ml)	1	13	14
Not demonstrable		0	81	81
Total		2	98	100

DISCUSSION

In healthy infants Andersen *et al* (2) and Felix *et al* (3) showed a frequency of CRP in serum increasing to above 50% during the first week of life With increasing age Felix *et al* found CRP in decreasing frequencies down to 2% in healthy infants 1–6 months of age With the more sensitive and accurate technique used in this study CRP could be demonstrated in 17 (17%) of the infants apparently healthy on their visit to the baby wellfare clinic Thirteen of these (13) only had CRP in trace amounts This group included four infants in whom the positive finding could presumably be related to a recent upper respiratory infection The only case where CRP had not vanished on reexamination turned out to have kidney abnormalities and pyelonephritis

In a random sample of 50 year-old men CRP was demonstrable in a frequency of 75% with the methods used in this study mostly only in trace amounts however (8) In a group of blood donors lower frequencies of sera containing trace amounts of CRP were found with lower age (6) In the infants of this study CRP was demonstrable in 19% These CRP-positive sera usually only contained trace amounts and at present this cannot be related to any clinical findings but for the few cases with recent upper respiratory infections

The results from this material of healthy infants may be used as a basis for a study of the clinical implications of the CRP concentration in diseased infants Such a study has been performed (9)

SUMMARY

Using sensitive and accurate immunodiffusion methods CRP could be demonstrated in 17 of 98 infants apparently healthy upon clinical examination Thirteen of these sera only contained trace amounts of CRP (0 5–1 μg/ml) The only case which had quantifiable amounts of CRP and which did not become negative upon control turned out to have kidney abnormalities and pyelonephritis

REFERENCES

1 Anderson H C & MacCarty M Determination of C reactive protein in the blood as a measure of the activity of the disease process in acute rheumatic fever *Amer J Med 8* 445 1950

2 Andersen H J Hanson, L A & Nilsson, L A The presence of C reactive protein in the neonatal period correlated to clinical conditions *Acta Path Microbiol Scand 54* 349 1962 (Abstract)

3 Felix N S Nakajima, H & Kagan B M Serum C reactive protein in infections during the first six months of life *Pediatrics 37* 270 1966

4 Nakamura R M Magsaysay R Ford J & Kanetake G M Significant variations in commercial C reactive protein antisera *Amer J Clin Path 44* 290 1965

5 Nilsson L A Comparative testing of precipitation methods for quantitation of C reactive protein in blood serum *Acta Path Microbiol Scand 73* 129 1968

6 — C reactive protein in apparently healthy individuals (blood donors) related to age *Acta Path Microbiol Scand 73* 619 1968

7 Nilsson L A & Hanson L A Studies on C reactive protein I Demonstration of C reactive protein by precipitation in capillary tubes and by precipitation in agar gel *Acta Path Microbiol Scand 54* 335 1962

8 Nilsson, L A & Tibblin G C reactive protein in a random sample of Swedish men aged fifty Distribution and relation to clinical manifestations *Acta Med Scand 183* 467 1968

9 Saxstad J Nilsson L A & Hanson L A C reactive protein in serum from infants as determined with immunodiffusion techniques II Infants with various infections In manuscript

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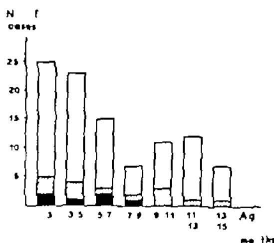


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Table 1 *Serum amino acid levels (μmoles/l)*

Age (days)	3	6	22	Normal range: Newborn 1–3 days	Normal range: Infants*
Daily protein intake (g/kg b w)	1 5	0 2	3.2		
Taurine	360	300	210	74–216	19–91
Threonine	1890	730	240	114–335	
Glutamic acid	450	460	550	20–107	46–290
Glycine	860	810	950	224–514	56–308
Alanine	500	420	260	236–410	99–313
Valine	450	720	270	80–246	57–262
Methionine	Trace	80	40	9 41	3–39
Isoleucine	200	290	110	27 94	26–94
Leucine	380	350	270	47 103	45–155
Tyrosine	400	200	90	42–99	11–122
Phenylalanine		190	90	42 110	23–69
Ornithine	480	430	200	49 151	
Lysine	710	830	360	114 269	45–144
Histidine	270	260	70	49 114	
Arginine	210	260	60	22 88	

Dickinson *et al* Pediatrics 36 2, 1965 (3)
Soupart 1962 (15)

METHODS

Serum and urine samples for the amino acid analyses were collected before and during the dietary treatment and stored at −20 C from 2 weeks to 3 months before analyzed The pH of the urine samples varied between 5–6 For screening and semiquantitative purposes high voltage electrophoresis and paper chromatography were used Quantitative analyses were performed on a Technicon Amino Acid Analyser after precipitation of the serum proteins with sulfosalicylic acid (50 mg/ml serum) Threonine and glutamine were well separated in these analyses while serine and glutamine were not equally well separated Proline and hydroxyproline were not measured Due to the storage the serum values of glutamic acid are not reliable (3) The serum levels of amino acids are expressed as μmoles/liter and the urinary excretion as μmoles/mg of creatinine

RESULTS

In Table 1 are listed the serum amino acid values of the patient on days 3 6 and 22 The protein intake on the preceding days are also given On the third and sixth day of life the patient showed an almost generalized hyperaminoacidemia Most increased were the levels of glycine threonine lysine serine valine leucine isoleucine and glutamic acid On the 22nd day the pattern was changed with most of the serum amino acids within or slightly above the normal range Glycine was however still markedly elevated so were to a lesser extent also leucine and lysine

A high voltage electrophoresis and paper chromatography of urine collected when the infant was 24 hours old was normal for age Table 2 illustrates the urinary excretion of amino acid on days 3 6 and 18 On the 3rd and 6th day there was a slight generalized hyperaminoaciduria with glycine and lysine showing the most marked elevation The excretion of valine and threonine was moderately increased On the 18th day there was a more isolated increase in the excretion of glycine threonine valine lysine and ornithine

DISCUSSION

At birth the amino acid patterns of urine from our patient seemed to be normal as judged by semiquantitative methods The abnormal changes soon occurred however Already on the third day of life when the clinical symptoms became apparent, an almost generalized

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ABNORMAL PATTERNS OF URINE AND SERUM AMINO ACIDS IN METHYLMALONIC ACIDEMIA

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Methylmalonic acidemia (MMA-emia) is a recently discovered inborn error of metabolism independently described by Oberholzer *et al* (10) in a 6 year old girl and by Stokke *et al* (16) in a newborn infant. The disease is clinically characterized by acidosis, dehydration, lethargy and coma. High serum levels and a large urinary excretion of methylmalonic acid (MMA) are characteristic chemical findings.

MMA is normally formed by carboxylation of propionyl CoA. The latter compound arises from many sources. The amino acid isoleucine, threonine and methionine are probably the most important in quantity, but propionate is also formed from odd and branched chain fatty acids, thymine, β-alanine, uracil and cholesterol. It is uncertain whether valine is metabolized through propionyl CoA or to methylmalonyl CoA directly. Two different enzymatic steps are involved in the conversion of methylmalonyl CoA to succinyl CoA: a racemization of MMA CoA catalyzed by an isomerase, followed by an intramolecular rearrangement catalyzed by a coenzyme B_{12} dependent mutase. It has been suggested that the metabolic block in MMA-emia is found in the latter step (10), in some cases possibly due to a decreased action of the vitamin B_{12} cofactor (12).

A distinct finding in MMA-emia is high serum levels of glycine (11, 16). In this paper the amino acid disturbances in our case of MMA-emia are described, and the results are compared with those found in Hyperglycinemia.

CASE REPORT

In september 1967 a newborn girl developed acidosis with severe clinical symptoms and accumulation of large amounts of an unknown organic in serum and urine. This acid was identified as methylmalonic acid. We therefore named the disorder Methylmalonic acidemia.

The parents of the patient were unrelated. Two siblings, both girls, died in the neonatal period suffering from acidosis. They were both born post term. The patient appeared well at birth and was started on a regular feeding schedule with cow's milk providing up to 4 g of protein and 200 calories on the second and third day. An acidosis with a standard bicarbonate of 14 mEq/l developed on the second and third day, and sodium bicarbonate had to be given. The family history and the clinical picture arose suspicion of an inborn error of metabolism. In order to try to improve the general condition of the child, the protein intake was reduced considerably from the 4th to the 6th day, and the patient was maintained mainly on i.v. fluids. However, no clinical improvement occurred. From the 6th day on the daily protein intake was gradually increased to 6–8 g or 3–4 g per kg of body weight. The body weight was steadily decreasing and the patient appeared dehydrated in spite of a fluid intake of about 200 ml/kg body weight daily.

After the accumulated organic acid had been identified as MMA, an attempt to treat the infant with a diet low in valine, isoleucine, threonine and methionine was performed. This treatment reduced the serum level of MMA from 30 to about 3 mg/100 ml and the daily urinary excretion of MMA from 1 g to 150 mg. Simultaneously the clinical condition improved considerably. However, the infant, who at that time suffered from an urinary tract infection, developed septicemia and died 6 weeks old.

We have previously pointed out that the clinical condition and the elevated amounts of glycine in blood and urine of our child with MMA-emia closely resembled the findings described in the ketotic form of Hyperglycinemia (12) We therefore proposed a relationship between the two diseases (4 16) The serum and urine amino acid patterns in Hyperglycinemia vary considerably with the severity of the disease Characteristic findings are increased serum levels of glycine serine leucine valine threonine isoleucine glutamic acid and probably also the basic amino acids (14) Correspondingly it has also been found increased urinary levels of glycine serine alanine phenylalanine lysine ornithine and histidine

In fact, recent reports confirm that some cases previously diagnosed as Hyperglycinemia actually are suffering from MMA-emia (8 9) On the other hand Rosenberg *et al* (11) and Morrow *et al* (9) did not find any accumulation of MMA in other patients with Hyperglycinemia Recent studies on leucocytes from a patient with ketotic hyperglycinemia showed a reduced oxidation of propionate to carbon dioxide while they could degrade methylmalonate normally (7) This indicates that ketotic hyperglycinemia may be divided into methylmalonic acidemia and propionic acidemia each of them consisting of different entities (12 16) It is more appropriate to use these terms instead of the non specific term hyperglycinemia

SUMMARY

Abnormal patterns of serum and urine amino acids were found in our patient with the recently discovered inborn error Methylmalonic acidemia The most distinct findings were increased serum and urine concentrations of lysine and glycine In addition the serum levels of leucine isoleucine valine threonine and glutamic acid and the urinary excretion of valine threonine and ornithine were increased Both the clinical picture and the amino acid patterns resemble those found in the ketotic form of Hyperglycinemia Recent studies indicate that hyperglycinemia consists of at least two separate diseases methylmalonic acidemia and propionic acidemia

REFERENCES

1 Armstrong N D Yates K N & Connelly J P Amino acid excretion of newborn infants during the first twenty four hours of life *Pediatrics 33* 975 1964

2 Childs B Nyhan W L Borden M Bard L & Cooke R E Idiopathic hyperglycinemia and hyperglycinuria A new disorder of amino acid metabolism *Pediatrics 27* 522 1961

3 Dickinson J C Rosenblum H & Hamilton P B Ion exchange chromatography of the free amino acids in the plasma of the newborn infant *Pediatrics 36* 2 1965

4 Halvorsen S Stokke O & Eldjarn L Methylmalonic acidemia/hyperglycinemia *Lancet I* 756 1968

5 Halvorsen, S Unpublished observations

6 Holt E L, Snyderman S F Norton P M, Roitman E & Finch J The plasma aminogram in Kwashiorkor *Lancet II* 1343 1963

7 Hsia Y E Scully K J & Rosenberg L E Defective propionate carboxylation in ketotic hyperglycinemia *Lancet I* 757 1969

8 Lindblad B Lindblad B S Olin P Svanberg B & Zetterstrom R Methylmalonic acidemia A disorder associated with acidosis hyperglycinemia and hyperlactatemia *Acta Paediat Scand* 57 417 1968

9 Morrow G Barness L A Auerbach, V H & DiGeorge A M Intermittent glycinemia and methylmalonic aciduria—a new syndrome *The Society for Pediatric Research Atlantic City 1968* Abstract p 20

10 Oberholzer V G Levin B Burgess E Ann & Young W Methylmalonic aciduria An inborn error of metabolism leading to chronic metabolic acidosis *Arch Dis Childh 42* 492 1967

11 Rosenberg L E Lilljeqvist, A-Ch & Hsia Y E Methylmalonic aciduria An inborn error leading to acidosis long-chain ketonuria and intermittent hyperglycinemia *New Eng J Med 278* 1319 1968

12 — Methylmalonic aciduria Metabolic block localization and vitamin B dependency *Science* 162 805 1968

13 Snyderman S E, Holt E L Norton P M Roitman, E & Phansalkar S V The plasma aminogram I Influence of the level of protein intake and a comparison of whole protein and amino acid diets *Pediat Res 2* 131 1968

14 Soriano J R, Taitz, L S, Finberg L & Edelmann C M, Jr Hyperglycinemia with ketoacidosis and leukopenia Metabolic studies on the nature of the defect *Pediatrics 39* 818 1967

Table 2 *Urinary amino acid excretion (μmoles/mg creatinine)*

Age (days)	3	6	18	Normal range for newborn
Daily protein intake (g/kg/b w)	1 5	0 2	3 0	
Taurine	2 02	1 25	0 78	0 20–7 64
Threonine	1 31	8 4	4 55	0 10–0 34
Glutamic acid	2 04	0 80	0 78	0–0 36
Glycine	15 7	24 5	35 6	0 16–4 46
Alanine	1 20	1 60	2 64	Trace –0 80
Valine	1 15	5 19	4 28	0–0 28
Methionine	Trace		0 51	0–0 18
Isoleucine		0 41		0–0 26
Leucine	0 74	1 69	0 01	Trace –0 14
Tyrosine	1 76	0 76	0 86	0–0 06
Phenylalanine	3 23	2 34	0 51	0–0 08
Ornithine	2 98	3 79	4 63	Trace –0 02
Lysine	14 7	18 4	12 09	0 08–0 64
Histidine	4 40	7 50	2 97	Trace –1 16
Arginine	0 55	0 84		0 02–0 30
Asparagine		5 05		0–0 12

Armstrong *et al* Pediatrics 33 975 1964 (1)

hyperaminoacidemia and hyperaminoaciduria were manifest

Later at the age of 3 weeks more isolated deviations from the normal patterns occurred In serum were found increased amounts of glycine, glutamic acid the branched acids isoleucine and leucine and the basic acids lysine and ornithine Increased amounts of glycine alanine threonine valine lysine and ornithine were excreted in urine

The high values for both serum and urine amino acids on the third and 6th day may indicate that the aminoaciduria was mainly of the overflow type After this time our data do not allow any conclusion whether the increased urinary amino acid levels were due to overflow or to a renal damage

The increased levels of valine threonine and isoleucine may easily be explained as these amino acids are precursors of MMA The increased concentration of glutamic acid at the expense of glutamine may be due to the storage of the serum and urine samples at −20 (see ref 3) The causes of the increased serum concentrations of glycine leucine and lysine are, however not known

Increased excretion of lysine has been observed in metabolic acidosis in the newborn and the amount may exceed the excretion of glycine which is normally the dominating amino acid also in this age period (5) In other acidotic cases the disturbances of the amino acid patterns have differed with the different underlying diseases It is therefore unlikely that the metabolic acidosis *per se* has led to the changes found in our patient

The weight of our patient was steadily decreasing in the period before the dietary treatment This loss of weight may be taken as a sign of a deranged protein metabolism The plasma aminograms of infants suffering from kwashiorkor show increased levels of glycine and serine while the levels of the branched amino acids and of tyrosine are depressed (6) The same pattern has been found in infants given a protein deficient diet for a few weeks (13) The abnormal amino acid picture of our patient may therefore only partly be caused by the general disturbance of the protein synthesis In addition the accumulated MMA or some of the preceding intermediates may have toxic effects on protein metabolism leading to high serum and urine concentrations of some of the amino acids

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FIBRINOLYSIS IN NEWBORNS

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Fibrinolytic activity in newborns has been studied since the beginning of the 1950s Since that time several papers have appeared on the subject

The interest in fibrinolysis during the neonatal period has hitherto been centred largely upon the assumption that a disturbance of fibrinolytic activity plays an etiological role in the hyaline membrane disease A comprehensive review has recently been given by Markarian *et al* (37) They stress the fact that the results of most investigators are at variance due to the use of different methods and nomenclature It is thus apparent that there is a need for further investigations of the fibrinolytic system in the newborn

This paper is concerned with an investigation of the fibrinolytic system in normal full term newborns at birth and during the first few days of life

CLINICAL MATERIAL AND BLOOD SAMPLING

The clinical material consisted of 107 fullterm normal newborns aged 0–5 days All were born in normal vertex presentation after uncomplicated pregnancy At 1 and 5 min all had an Apgar score of 8 9 or 10 Infants with visible jaundice were not accepted

The clamped cord was cut off under sterile precautions and a plastic catheter (Infant feeding tube French No 8) was introduced into the umbilical vein Blood was then allowed to run freely into test tubes This sampling technique was used throughout irrespective of the time after delivery Between 10–15 ml of blood was obtained from each infant but it was not always possible to analyse each sample by all methods Samples were obtained at the earliest 5 min after delivery *The material was arranged in* 10 age groups (Table 2) The oldest infant was 5 days and 20 hours

In 21 cases from the group 0–30 min samples were obtained from the placental side of the umbilical cord as well as from the catheterized umbilical vein for comparative studies Blood from the proximal end of the divided cord was allowed to run directly into test tubes Milking of the cord was avoided

Sampling was performed at the department of obstetrics All samples were transported as quickly as possible to the coagulation laboratory located in the close vicinity In order to get an accurate measure of the fibrinolytic activity it is important that the time lag between sampling and assay is as short as possible (4 14 22 56) In this study the time lag never exceeded 30 min

LABORATORY PROCEDURES

Collection of blood The blood was collected with the silicone technique and citrated plasma and serum were prepared as described previously (39 43)

The following determinations were made

1 *Fibrinolytic activity* on unheated and heated fibrin plates according to Andersson *et al* (4) Fibrinogen from one and the same batch was used for all the assays in this study The normal ranges on unheated plates for adults at our laboratory are 0–85 mm for plasma and 0–116 mm for the resuspended euglobulin precipitate Mean values 23 mm and 74 mm respectively There is no activity of plasma on heated plates in healthy adults

2 *Euglobulin clot lysis time* according to Nilsson & Olow (40 b) Normal value for adults is >100 min

3 *Fibrinolytic split products* in serum and in serum from blood collected with an inhibitor of fibrinolysis E aminocaproic acid (EACA) with an immunochemical method described by Nilehn (38) In this method an antiserum against the D fraction of the split products is applied to the agarose gel and

15 Soupart P Free amino acids of blood and urine in the human *In* J T Holden (ed) *Amino acid pools* Elsevier Amsterdam 1962
16 Stokke O Eldjarn L Norum K R Steen Johnsen J & Halvorsen S Methylmalonic acidemia A new inborn error of metabolism which may cause fatal acidosis in the neonatal period *Scand J Clin Lab Invest* *20* 313 1967

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5 *α_2-macroglobulin* according to an esterolytic method by Ganrot (25) The result is expressed in per cent and is related to a normal standard consisting of pooled serum from healthy military orderlies about 20 years of age The normal adult range is 80–120

6 *Antiplasmin* activity so-called progressive antiplasmin according to a modification of Shamash & Rimon's caseinolytic method (50) Glycerol activated human plasmin (Kabi 1375) was added to a 1 20 dilution of the serum and after 120 minutes incubation of the mixture at 37 C the residual plasmin was measured with casein as a substrate for the enzymatic digestion The result is expressed in arbitrary caseinolytic units (ACU) defined in such a way that 10 ACU are equal to the amount of enzyme producing an increase in optical density at 280 mμ of 0 300 using a 3 casein solution and a digestion time of 20 minutes The normal adult range is 400–600 ACU/ml

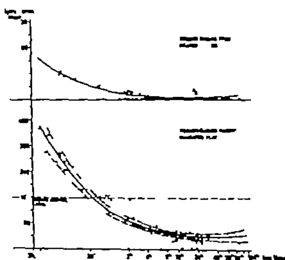


Fig 2 Fibrinolytic activity of resuspended euglobulin precipitate on unheated and heated fibrin plates in 161 infants Correlation between lysed area in mm (y) and logarithmed time after delivery (x) Since it was obvious that the values fitted badly to a straight line second degree polynomial regression was used The square root of y was chosen to stabilize the variance at different time periods The regression curve for unheated plates can be derived from the formula $\sqrt{y} = 27\ 45 - 12\ 67x + 1\ 88x^2$ The nonlinear shape of this curve is statistically significant (for both regression coefficients is $p < 0\ 001$) The broken lines represent the 95% confidence interval The regression curve for heated plates can be derived from the formula $\sqrt{y} = 12\ 28 - 6\ 89x + 1\ 12x^2$ Confidence interval was not calculated since there are many zero values

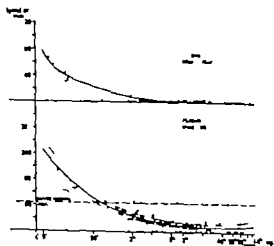


Fig 1 Fibrinolytic activity of plasma on unheated and heated fibrin plates in 161 infants Correlation between lysed area in mm (y) and logarithmed time after delivery (x) Since it was obvious that the values fitted badly to a straight line second degree polynomial regression was used The square root of y was chosen to stabilize the variance at different time periods The regression curve for unheated plates can be derived from the formula

$\sqrt{y} = 27\ 9 - 11\ 14x + 1\ 64x^2$

The nonlinear shape of this curve is statistically significant (for both regression coefficients is $p < 0\ 001$) The broken lines represent the 95% confidence interval The regression curve for heated plates can be derived from the formula

$\sqrt{y} = 13\ 34 - 7\ 0$[illegible]$x + 0\ 90x^2$

The nonlinear shape of this curve is statistically significant (for both regression coefficients is $p < 0\ 001$) Confidence interval was not calculated because of many zero values

7 *Inhibitors of the urokinase activation of plasminogen in serum* according to a clot method described by Paraskevas *et al* (43) As pointed out by these authors an extremely high or low content of antiplasmin in the serum will interfere with the method The result is expressed in per cent and is related to a normal standard consisting of pooled serum from 25 healthy military orderlies about 20 years of age The normal adult range is 60–140%

8 *Fibrinogen* according to a modification of Jacobsson's method as described by Nilsson & Olow (40 a) in which the blood is collected in tubes with and without an inhibitor of fibrinolysis E aminocaproic acid (EACA) Only the values for blood collected with EACA are given

9 The *total serum protein* was measured by the biuret method

10 The venous *haematocrit* was estimated by the macromethod

Table 1 *Fibrinolytic activity in umbilical vein blood*

Correlations between activity on fibrin plates fibrinolytic split products and euglobulin clot lysis time

Fibrin plates	Split products Serum	Serum EACA	Euglobulin clot lysis time
Plasma			
Unheated plates	135* $r=+0.65$ $p<0.001$	—	—
Heated plates	—	137* $r=+0.37$ $p<0.001$	—
Resusp euglob precipitate Unheated plates	135* $r=+0.73$ $p<0.001$	—	156* $r=-0.87$ $p<0.001$
Heated plates	—	137 $r=+0.32$ $p<0.001$	

* No of subjects

the high molecular weight substances (HMWS) serve as standard

4 *Plasminogen* according to a modification of the immunochemical method by Ganrot & Niléhn (27) To avoid activation of plasminogen *in vitro* the blood was collected in tubes containing EACA We used a specific rabbit antiserum to human plasminogen obtained by immunising rabbits weighing 2 5–3 kg with plasminogen (Grade A freeze dried 25 Sgouris CU/ampoule from AB Kabi Stockholm) The determination was made according to Laurell (36) with electrophoresis in agarose gel containing the specific antiserum in dilution 1 200 10 microlitres of each serum sample diluted 1 5 was applied and electrophoresis was allowed to continue for 4 hours at a voltage of 20 V/cm Mixed serum from 20 healthy volunteers served as reference The height of the peaks obtained is a measure of the amount of antigen in the samples Plasminogen and plasmin have the same immunological determinants so that the result of the assay is a measure of the sum of plasminogen and plasmin in the sample

A comparison was made between determinations of the plasminogen in samples collected in tubes without and with addition of EACA from 20 healthy men and 20 healthy non pregnant women not using oral contraceptives aged 20–50 years The plasminogen level in the samples with EACA was significantly lower than in samples without EACA ($p<0.01$) The standard deviation of the plasminogen in the former samples was also smaller (16% and 21 respectively) With the method where EACA was used there was no significant difference between men and women ($p>0.1$) The range of variation calculated with 2 SD in these 40 persons was 61–125% with a mean of 93

Table 2 *Results of determinations of plasminogen inhibitors total serum protein and venous haematocrit in umbilical vein blood*

	Range normal adults	Mean	Range	SD	No of infants/time after delivery in hours Total	<1/2	1/2–2	2–4	4–8	8–12	12–24	24–36	48–72	72–96	96–140	Correlation to time after delivery r	p
Plasminogen immunol ( )	61–125	43 4	25–130	16 0	162	45	19	25	15	14	23	9	5	5	2	+0 05	>0 5
α macroglobulin ( )	80–120	169 7	78–291	37 3	161	44	19	23	16	12	25	10	5	5	2	−0 04	>0 5
Antiplasmin — ACU/ml	400–600	478 9	340–516	79 6	31	19	3	7	2	—	—	—	—	—	—	−0 14	>0 5
Inhibitors of plasminogen activation ( )	60–140	183 7	84–490	76 2	148	45	15	17	14	10	24	11	6	4	2	−0 10	0 2
Total serum protein (g/100 ml)	6 6–8 0	7 43	5 9–8 4	0 6	148	36	22	15	16	13	24	11	6	5	2	−0 16	0 04
Venous haematocrit ( )	♂ 43 5 ♀ 39 2	52 4	43–62	10 5	66	22	—	—	3	3	17	9	5	5	2	+0 16	0 2

tubes with EACA contained only small amounts of split products from the very beginning *vi* 0–7 mg/100 ml (mean 1 2 mg/100 ml) and no measurable amounts after 8 hours of age Statistical analysis of the results is presented in the legend to Fig 4

There was a good agreement between the three methods used for measuring fibrinolytic activity Table 1 shows certain correlations between the activity on fibrin plates split products and euglobulin clot lysis time The correlation between the activity of plasma and resuspended euglobulin precipitate, respectively and split products in serum was statistically significant as was the correlation between activities on heated plates and split products in serum obtained in the presence of EACA ($p < 0.001$) The correlation between the activity of resuspended euglobulin precipitate and euglobulin clot lysis time was also statistically significant ($p < 0.001$)

Plasminogen (Table 2) The level was on the average 43 4 (range 25–130) of the normal adult value The correlation with time after delivery was not statistically significant

α macroglobulin (Table 2) was moderately higher throughout the groups compared to the normal adult value The mean was 169 7 with a range of 78–291

Inhibitors of plasminogen activation (Table 2) A higher level than in normal adults was found with a mean of 183 7 (range 84–490)

Antiplasmin (Table 2) could be assayed in only 31 cases because of shortage of serum The results obtained fell within the normal range for adults with a mean of 478 9 ACU/ml (range 340–516 ACU/ml)

The correlation between the values for the different inhibitors to time after delivery was not statistically significant

Fibrinogen (Fig 5) The mean value for the first 24 hours bordered to the lower normal range for adults (0 26 g/100 ml) with a relatively wide spread (0 13–0 43 g/100 ml) On the second day there was a tendency to higher

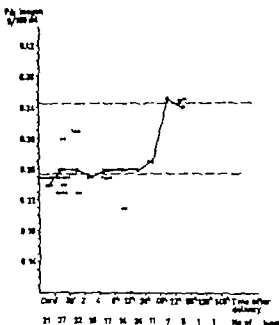


Fig 5 Fibrinogen values (g/100 ml) in cord blood (21 subjects) and in umbilical vein blood (155 subjects) The unbroken line represents the mean values There is a significant increase in the mean value on the third day of life ($p < 0.01$) The broken lines represent the adult normal range

values with a mean of 0 27 g/100 ml and a range of 0 20–0 36 g/100 ml From the third day on the values were significantly higher ($p < 0.01$) with a mean of 0 35 g/100 ml (range 0 24–0 44 g/100 ml)

Total serum protein (Table 2) was checked in 148 cases and agreed with reported normal values for the newborn period (51) The mean value for the whole material was 7 4 g/100 ml (range 5 9–8 4 g/100 ml) There was a small but significant decrease with increasing time after delivery

Haematocrit values (Table 2) were obtained in 66 cases and agreed with those reported by Oski & Naiman (42) but were lower than those of Usher *et al* (57) The mean value for all cases was 52 4 with a range from 43 to 62 There was no significant correlation to time after delivery but unfortunately no determinations were made between 30 minutes and 4 hours after parturition when the plasma volume is known to decrease (57)

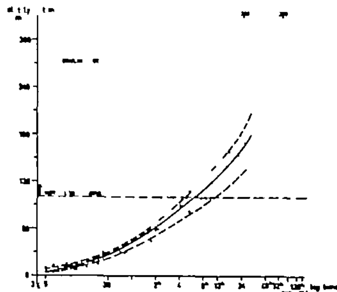


Fig 3 Euglobulin clot lysis times in 155 infants Correlation between clot lysis time (y) and logarithmed time after delivery (x) Since it was obvious that the values fitted badly to a straight line second degree polynomial regression was used The regression curve can be derived from the formula log y = 0 06 + 1 13x–0 14x The nonlinear shape of this curve is statistically significant (for both regression coefficients is $p < 0.001$) The broken lines represent the 95% confidence interval

RESULTS

Umbilical vein blood

Fibrinolytic activity on fibrin plates The results are given in Figs 1 and 2 In samples obtained within half an hour of parturition both the plasma and the resuspended euglobulin precipitate contained considerable fibrinolytic activity as measured on unheated plates Values between 31–355 mm (mean 180 mm) were noted for plasma and between 93–425 mm (mean 260 mm) for the resuspended euglobulin precipitate The activity successively fell, and after 4 hours it was within the normal range for adults

There was also a plasmin activity as measured on heated plates In the group where the samples were taken within the first half hour of parturition, the activity was 18–121 mm (mean 50 mm*) for plasma and 14–99 mm (mean 50 mm) for the resuspended euglobulin precipitate The plasmin activity fell rapidly and was no longer demonstrable in samples collected 4 hours after parturition

In all four measurements of fibrinolytic activity (plasma and resuspended euglobulin precipitate on unheated and heated plates) the correlation between lysed area and the time after delivery was statistically significant as is noted in the legends to Figs 1 and 2

Euglobulin clot lysis time (Fig 3) In samples obtained within half an hour of delivery the mean value found was 13 minutes The lysis times then became successively longer and already 4 hours after parturition they fell within the normal range for adults (> 100 min) Statistical analysis of the findings is presented in the legend to Fig 3

Fibrinolytic split products (Fig 4) Serum from sample collected within the first half hour of parturition contained split products 2–70 mg/100 ml with a mean of 16 mg/100 ml The amounts then decreased with increasing interval between parturition and sampling From 8–12 hours and on split products, if any were demonstrable in only small amounts (<4 mg/100 ml)

Serum prepared from blood collected in

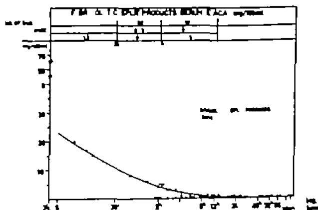


Fig 4 Fibrinolytic split products in serum (174 infants) and serum with EACA (168 infants) Correlation between split products in serum (y) and logarithmed time after delivery (x) Since it was obvious that the values fitted badly to a straight line second degree polynomial regression was used The regression curve can be derived from the formula y = 39 88–26 13x + 4 28x The nonlinear shape of this curve is statistically significant (for both regression coefficients is $p < 0.001$) Confidence interval was not calculated because of many zero values after about 8 hours For split products in serum with EACA range and mean values in the different age groups are given The correlation with time after delivery is statistically significant ($r = -0.45$ $p < 0.001$)

tubes with EACA contained only small amounts of split products from the very beginning viz. 0–7 mg/100 ml (mean 1 2 mg/100 ml) and no measurable amounts after 8 hours of age Statistical analysis of the results is presented in the legend to Fig 4

There was a good agreement between the three methods used for measuring fibrinolytic activity Table 1 shows certain correlations between the activity on fibrin plates split products and euglobulin clot lysis time The correlation between the activity of plasma and resuspended euglobulin precipitate respectively and split products in serum was statistically significant as was the correlation between activities on heated plates and split products in serum obtained in the presence of EACA ($p<$ 0 001) The correlation between the activity of resuspended euglobulin precipitate and euglobulin clot lysis time was also statistically significant ($p<0\ 001$)

Plasminogen (Table 2) The level was on the average 43 4 (range 25–130) of the normal adult value The correlation with time after delivery was not statistically significant *α macroglobulin* (Table 2) was moderately higher throughout the groups compared to the normal adult value The mean was 169 7 with a range of 78–291

Inhibitors of plasminogen activation (Table 2) A higher level than in normal adults was found with a mean of 183 7 (range 84–490)

Antiplasmin (Table 2) could be assayed in only 31 cases because of shortage of serum The results obtained fell within the normal range for adults with a mean of 478 9 ACU/ml (range 340–516 ACU/ml)

The correlation between the values for the different inhibitors to time after delivery was not statistically significant

Fibrinogen (Fig 5) The mean value for the first 24 hours bordered to the lower normal range for adults (0 26 g/100 ml) with a relatively wide spread (0 13–0 43 g/100 ml) On the second day there was a tendency to higher

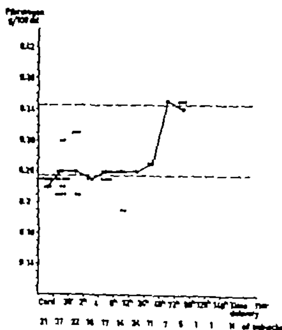


Fig 5 Fibrinogen values (g/100 ml) in cord blood (21 subjects) and in umbilical vein blood (155 subjects) The unbroken line represents the mean values There is a significant increase in the mean value on the third day of life ($p<0\ 01$) The broken lines represent the adult normal range

values with a mean of 0 27 g/100 ml and a range of 0 20–0 36 g/100 ml From the third day on the values were significantly higher ($p<0\ 01$) with a mean of 0 35 g/100 ml (range 0 24–0 44 g/100 ml)

Total serum protein (Table 2) was checked in 148 cases and agreed with reported normal values for the newborn period (51) The mean value for the whole material was 7 4 g/100 ml (range 5 9–8 4 g/100 ml) There was a small but significant decrease with increasing time after delivery

Haematocrit values (Table 2) were obtained in 66 cases and agreed with those reported by Oski & Naiman (42) but were lower than those of Usher *et al* (57) The mean value for all cases was 52 4 with a range from 43 to 62 There was no significant correlation to time after delivery but unfortunately no determinations were made between 30 minutes and 4 hours after parturition when the plasma volume is known to decrease (57)

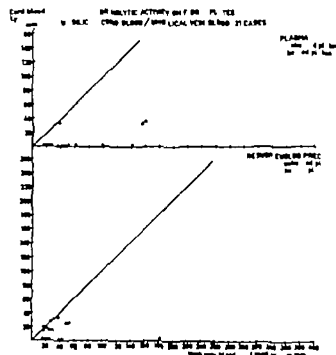


Fig 6 Comparison between fibrinolytic activity on fibrin plates of cord blood and umbilical vein blood 21 cases

activator and plasmin activity in the umbilical vein blood than in cord blood with a range of 100–355 mm for plasma and 182–373 mm for resuspended euglobulin precipitate In cord blood the range was 0–146 mm for plasma and 0–249 mm for resuspended euglobulin precipitate

The *euglobulin clot lysis times* were also significantly shorter for umbilical vein blood (5–20 min) than for cord blood (10–330 min) The mean difference was 59 min ($p<0\,001$)

The split products in serum from umbilical vein blood were present in significantly larger quantities (8–50 mg/100 ml) than in serum from cord blood (0 30 mg/100 ml) The mean difference was 12 6 mg/100 ml ($p<0\,01$)

In serum obtained in the presence of EACA split products were demonstrated in 7 cases from umbilical vein blood and in 5 cases from cord blood The highest values were 5 mg/100 ml and 3 mg/100 ml respectively without significant differences between the groups

As to *plasminogen α -macroglobulin* and *fibrinogen* no significant differences were obtained

Inhibitors of plasminogen activation were significantly higher in the umbilical vein blood than in cord blood The mean difference was 38 4 ($p<0\,05$)

Total serum protein and *venous haematocrit* were significantly higher in the umbilical vein

B *Comparison between umbilical vein blood and cord blood*

In 21 cases a comparison was made between the fibrinolytic activity and all the other above mentioned factors in cord blood and umbilical vein blood obtained 5–22 minutes after parturition The results are shown in Fig 6 and Table 3

Fibrinolytic activity on fibrin plates showed a significantly higher ($p<0\,001$) plasminogen

Table 3 *Comparison between umbilical vein blood (V) and cord blood (C)*

	No of subjects	V		C		Mean difference between V and C	P
		Range	Mean	Range	Mean		
Plasminogen immunol ( )	14	27–130	59 7	40–103	59 7	± 0	>0 05
α_2 macroglobulin ( )	16	127–253	178 1	74–291	163 5	+14 6	>0 05
Inhibitors of plasminogen activation ( )	18	101–259	180 8	64–258	142 7	+38 1	<0 05
Euglobulin clot lysis time (min)	21	5–20	12	10–330	71	−59	<0 001
Fibrinolytic split products (mg/100 ml)							
(a) serum	19	8–50	21 3	0–30	8 6	+12 7	<0 01
(b) serum — EACA	20	0–5	1 1	0–3	0 5	+ 0 6	>0 05
Fibrinogen (g/100 ml)	20	0 17–0 37	0 25	0 11–0 37	0 24	+ 0 01	>0 05
Total serum protein (g/100 ml)	16	6 2–8 3	7 4	5 9–8 0	6 9	+ 0 5	<0 001
Haematocrit ( )	14	45–59	52	41–60	48 7	+ 3 3	<0 01

blood than in cord blood ($p<0.001$ and <0.01 respectively) which is also in agreement with earlier investigations already cited above

DISCUSSION

Numerous investigations of the fibrinolytic activity in newborns have been published up to date Most of these studies report results obtained with cord blood (5 7 10 11 16 24 30 31 33 34 44 48 53) Some investigators have studied blood obtained by puncture of the cord vein and/or artery and thereby avoided any admixture of thromboplastic material from umbilical cord tissue (8 14 17 18 35 46) Venipuncture of the infant has been used particularly when serial sampling was performed in the first days of life (8 10 11 13 23 24 28 37) Only in three investigations of term infants (2 20 21) has blood been obtained by catheterisation of the umbilical vessels of the infant

The fibrinolytic activity has been measured by many different methods (e g clot lysis of whole blood plasma or englobulin fraction measuring of the fibrinogen after different times of incubation thromboelastogram fibrin plates) The results are therefo e not s rictly comparable On the whole the fibrinolytic activity in the newborn is reported to be higher than that in normal adults or the mother but Gaburro et al (24) found lower activity in newborns than in their mothers Chaplin (14) and Cope & Simmons (18) found the activity in umbilical artery blood to be higher than that in umbilical vein blood Serial sampling has shown decreasing fibrinolytic activity with increasing interval after delivery (10 21 23 28 37)

Some investigations (45 49) have been carried out on serum which however gives information only on the plasminogen and inhibitors but not on activator activity

In the evaluation of the results two very important factors capable of influencing the plasminogen activator activity measured should be borne in mind namely the interval between sampling and analysis and the temperature of the sample before analysis Fearnley (22) Truelove (56) Chaplin (14) and later Nilsson & Olow (40 b) stressed that investigation of the activator activity should be performed within half an hour of sampling Truelove found that cooling of the blood to +4 C rather increased the activator activity while Nilsson & Olow (40 b) showed that freezing reduced it Berglund (10) who was the first to study the neonatal fibrinolytic activity on fibrin plates found such activity in only 50% of her series but at the same time pointed out that her results might be falsely low owing to the interval between sampling and analysis Ambrus et al (2 3) found only traces of activator activity in healthy fullterm newborns but they did not mention the interval between sampling and analysis

In our investigation which was performed throughout on blood from the catheterised umbilical vein of the infant the fibrinolytic activity was determined in plasma and in the resuspended englobulin precipitate

Our results show that all healthy fullterm newborns have a plasminogen activator activity which at birth is much higher than that in blood from normal adults The activity falls to the level of adults within about 4 hours of birth We also found plasmin activity which may however not be representative of the situation in vivo but due to activation of plasminogen in vitro This assumption is strengthened by the fact that fibrinolytic split products were demonstrated mainly in blood obtained without EACA and only in at most small amounts in the blood collected in tubes containing EACA

A significant correlation was found between the results obtained by the methods used fibrin plates englobulin clot lysis time and determination of the fibrinolytic split products in serum without addition of EACA The englobulin clot lysis time is a rather crude measure of the fibrinolytic activity and gives reliable results only when considerable fibrino-

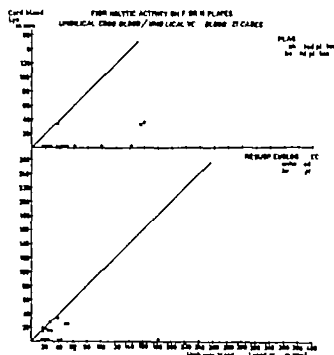


Fig 6 Comparison between fibrinolytic activity on fibrin plates of cord blood and umbilical vein blood 21 cases

B *Comparison between umbilical vein blood and cord blood*

In 21 cases a comparison was made between the fibrinolytic activity and all the other above mentioned factors in cord blood and umbilical vein blood obtained 5–22 minutes after parturition The results are shown in Fig 6 and Table 3

Fibrinolytic activity on fibrin plates showed a significantly higher ($p<0.001$) plasminogen activator and plasmin activity in the umbilical vein blood than in cord blood with a range of 100–355 mm for plasma and 182–373 mm for resuspended euglobulin precipitate In cord blood the range was 0–146 mm for plasma and 0–249 mm for resuspended euglobulin precipitate

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of an inhibitor of fibrinolysis (EACA) which was important because of the high fibrinolytic activity during the first hours after birth. The values therefore reflect the situation *in vivo*. Inhibitors of fibrinolysis have not previously been used in the determination of fibrinogen in the newborn. Reported low values might therefore be due to fibrinogenolysis *in vitro* and normal values might be due to sampling from the cord with lower fibrinolytic activity. But even with the addition of EACA the spread in our series was relatively wide. About 50 % of the values in the cord blood and in the vein blood within the first day fell below the lower border of normal adult range (Fig. 5). There was no significant difference between cord blood and umbilical vein blood obtained within 30 min after delivery. At 3 days of age the mean value was significantly higher compared to that for the first 2 days ($p < 0.01$). This difference is in agreement with some earlier studies. Taylor (54) reported an insignificant rise in the mean concentration of fibrinogen during the first several days of life. Fisher *et al.* (23) found the difference to be significant with values in the lower normal range in the first day and definitely normal levels on the 4th day. Recently Kanitzky *et al.* (32) have reported on immunological fibrinogen assays in healthy prematures repeatedly studied during the first ten days. They found higher levels already at 4 hours of age and then successively increased values the following days. They conclude that this increase can not be due solely to changes in plasma volume but must be the result of increasing synthesis of fibrinogen. Usher *et al.* (57) have shown that the important shifts in plasma volume of the newborn occur within the first 24 hours with a minimum plasma volume at 4 hours. At 24 hours the plasma volume has stabilised and no significant changes occur during the following days. An increase in fibrinogen concentration at 4 hours might in part be due to the plasma concentration and/or a diminished fibrinogenolysis at that time. Higher values after the first day, however, can be explained neither by alterations in plasma volume nor in fibrinolytic activity but speak in favour of an accelerated synthesis.

The fibrinolytic activity develops already during intra-uterine life (17, 19, 58) and probably serves to maintain a free foeto-placental circulation (17). The actual stress of delivery may also increase this activity which afterwards rapidly decreases. The question remains whether the increased fibrinolytic activity in the newborn serves any physiological purpose. Its function might be to prevent deposition of fibrin in the alveoli as was first proposed by Phillips & Skrodelis (44). A disturbance of the fibrinolytic system has been regarded as one factor in the complex pathogenesis of the development of hyaline membranes in prematures (2). Pulmonary ischaemia has been suggested as another important factor (15). In the light of Astrup's observation (6) that the content of the plasminogen activator activity in a tissue is related to its vascularisation and is thus high in well vascularised organs, one might imagine that a decreased circulating activator activity in the ischaemic lungs in prematures is a contributory cause of the development of hyaline membranes.

SUMMARY

The fibrinolytic system was studied in 207 normal fullterm newborns during the first 5 days of life. A high but rapidly decreasing plasminogen activator activity was found during the first 4 hours in umbilical vein blood in all of the children studied. The activity in the cord blood was lower and cannot be regarded as representative of the child. Fibrinolytic split products were demonstrated in large and rapidly decreasing amounts in serum samples without the addition of EACA but in at most small amounts in serum when EACA was added at sampling. These findings confirm the high plasminogen activator activity at birth which rapidly falls and furthermore show that there are no significant amounts of split products *in vivo* in the newborn. The plasminogen

lysis is present e g when the lysis time is less then 100 minutes

The activity of the cord blood was low compared to that of umbilical vein blood, or even absent This difference also reported by Markarian *et al* (37) probably explains why earlier investigations on umbilical cord blood could not always demonstrate fibrinolytic activity Sampling of blood from the cut cord or puncture of the umbilical cord vein have given the same results This makes it less likely that some possible inhibitory substance in Wharton s jelly might lower the fibrinolytic activity in cord blood by contamination

The difference found in activity between the cord blood and umbilical vein blood can probably be explained by production of activator by the newborn Many data discussed by Beller *et al* (8) and Markarian *et al* (37) strengthen this assumption At delivery the mother has low fibrinolytic activity (8) the placenta tissue at term is fibrinolytically inactive (1 9) and the activity is higher in umbilical artery blood than in vein blood (14 18) The stress produced by the delivery may also possibly increase the activator activity

Fibrinolytic split products in cord blood have earlier been demonstrated by Stiehm (52) Bonifaci *et al* (12) and Hathaway *et al* (29) by use of precipitin tube technique or immunoelectrophoresis and with an antiserum to fibrinogen As far as we can judge these authors did not add inhibitors of fibrinolysis when collecting the blood samples The advantage of the method used by us has been discussed by Nilehn (38)

The findings of large and rapidly decreasing amounts of split products in serum samples without the addition of EACA and only small amounts of split products in serum when EACA was added at sampling speak clearly in favour of a high plasminogen activator activity at birth which rapidly falls Our opinion on this point is at variance with that of Bonifaci *et al* (12)

Our determination of plasminogen by the immunochemical method where blood is collected in the presence of EACA in order to prevent plasminogen activation in vitro has not previously been used in newborns We found the values to be about 50 of the normal adult level This is in agreement with earlier investigations with other methods (2 3 8 13 17 41 44 45 49 55) Our results suggest that the plasminogen level is due to a low content of plasminogen in the infant and not to increased activation to plasmin after sampling At variance with Beller *et al* (8) we found no significant difference between the plasminogen content of cord blood and umbilical vein blood

No unanimity has been achieved regarding the inhibitors of fibrinolysis in the newborn Many earlier investigations report lower values than in adults (35 45 47 48 49) or in the mother (44) while others have found normal (2 8) or higher levels (13 54) than in adults Apart from Beller *et al* (8) previous workers have not been able to distinguish between inhibitors of plasminogen activation and antiplasmin because in their methods they used plasmin which had been activated by streptokinase or urokinase

We determined the inhibitors of plasminogen activation α -macroglobulin and progressive antiplasmin Inhibitors of plasminogen activation and α -macroglobulin were moderately higher than the normal adult value Ganrot & Scherstén (26) have already shown that α macroglobulin is increased in umbilical cord blood Using Ganrot s method we found the same values in the umbilical vein blood

The antiplasmin method used excludes inhibitors of plasminogen activation because the plasmin used was activated with glycerol and not with streptokinase or urokinase We found the values to be within the range for adults The newborn was thus found to have a higher total inhibitor level than normal adults The high activator activity cannot be ascribed to a low inhibitor content The function of the high inhibitor level may presumably be to counterbalance the high activator activity

Fibrinogen was determined in the presence

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level was decreased in accordance with previous investigations The high activator activity is counterbalanced by adequate inhibitory protection with an increase of the α macroglobulin and inhibitors of plasminogen activation and the adult content of antiplasmin The mean fibrinogen values in the first 48 hours of life are in the lower part of the normal range but increase on the third day to a significantly higher level

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REFERENCES

1 Albrechtsen O K Fibrinolytic activity in the organism *Acta Physiol Scand 47* Suppl 165 1959

2 Ambrus C M Weintraub D H Dunphy D Dowd J E Pickren J W Niswander K R & Ambrus J L Studies on hyaline membrane disease I The fibrinolysin system in pathogenesis and therapy *Pediatrics 32* 10 1963

3 Ambrus C M Weintraub D H Niswander K R & Ambrus J L Studies on hyaline membrane disease II The ontogeny of the fibrinolysin system *Pediatrics 35* 91 1965

4 Andersson L Nilsson I M & Olow B Fibrinolytic activity in man during surgery *Thromb Diathes Haemorrh 7* 391 1962

5 Aristowicz Z Euglobulin fibrinolysis in newborn infants healthy prematures and prematures with respiratory distress syndrome (R D S) *Ann Paediat 206* 265 1966

6 Astrup T Tissue activators of plasminogen *Fed Proc 25* 42 1966

7 Beller F K *Die Gerinnungsverhaltnisse bei der Schwangeren und beim Neugeborenen* Barth Leipzig 1957

8 Beller F K Douglas G W & Epstein M D The fibrinolytic enzyme system in the newborn *Am J Obstet Gynec 96* 977 1966

9 Beller F K Goessner W & Herrschlein H J Tissue activator of the fibrinolytic system in placental tissue *Obstet Gynecol 20* 117 1962

10 Berglund G The fibrinolytic activity in the newborn *Acta Paediat Scand 47* 511 1958

11 Bernardi M Grasso E Nasso S Verga A & Albanesi R Sulla patogenesi delle emorragie del neonato *Il Lattante 24* 219 1953

12 Bonifaci E Baggio P & Gravina E Demonstration of split products of fibrinogen in the blood of normal newborns *Biol Neonat 12* 29 1968

13 Bruster H & Pfitzner W Untersuchungen uber das fibrinolytische Potential bei reifgeborenen und jungen Sauglingen *Mschr Kinderheilk 113* 476 1965

14 Chaplin H Observations on fibrinolysis in umbilical cord blood *Clin Sci 13* 111 1954

15 Chu J Clements J A Cotton E K Klaus M H Sweet A Y & Tooley W H Neonatal pulmonary ischemia Part I Clinical and physiological studies *Pediatrics 40* 709 1967

16 Ciulla U & Luraschi C L attivita fibrinolitica del siero di sangue del funicolo ombelicale *Ann Obstet Ginec 76* 207 1954

17 Cope I & Mitchell P The plasminogen-plasmin content of umbilical cord blood *Aust N Z J Obst Gynaec 4* 117 1964

18 Cope I & Simmons E The fibrinolytic activity of umbilical cord blood *J Obst & Gynaec Brit Emp 65* 416 1958

19 Ekelund H Hedner U & Astedt B Fibrinolysis in the foetus To be published

20 Engstrom L & Karger L Changes in plasma fibrinolytic activity of newborn infants during first hour after birth *Acta Paediat Scand 53* 326 1964

21 — Fibrinolytic activity in plasma of newborn infants *Acta Paediat Scand 53* 379 1964

22 Fearnley G R Revill R & Moore Tweed J Observations on the inactivation of fibrinolytic activity in shed blood *Clin Sci 11* 309 1952

23 Fisher Sh Schwartz M Gottlieb A Ben Yoseph N & Shapiro S Fibrinolysis fibrinogen and factor XIII in newborn infants *Thrombos Diathes Haemorrh 20* 542 1968

24 Gaburro D Panizza G F & Bonifaci E Les alterations du système fibrinolytique chez le nouveau né *Ann de Pediatrie 38* 621 1962

25 Ganrot P O Determination of α_2-macroglobulin as trypsin protein esterase *Clin Chim Acta 14* 493 1966

26 Ganrot P O & Scherstén B Serum α_2-macroglobulin concentration and *its variation with age* and sex *Clin Chim Acta 15* 113 1967

27 Ganrot P O & Niléhn J E Immunochemical determination of human plasminogen *Clin Chim Acta 22* 335 1968

28 Grossman B J Heyn R M & Rozenfeld I H Coagulation studies in the newborn infant I Normal infants *Pediatrics 9* 182 1952

29 Hathaway W E Mull M M & Pechet G S Disseminated intravascular coagulation in the newborn *Pediatrics 43* 233 1969

30 Howell W L Fibrinolytic activity of the umbilical cord blood *Med Ann D C 29* 491 1960

31 Jenny J & Dockert F Die Gerinnungsverhaltnisse beim lebensfrischen nichtasphyktischen Neugeborenen nach normaler Geburt und Schwangerschaft Gynaecologia 155 175 1963
32 Karitzky D Pringsheim W Zimmer O & Kunzer W Veranderungen der Fibrinogenkonzentration im Plasma gesunder Fruhgeborener am I Lebenstag Z Kinderheilk 105 73 1969
33 Kunzer W & Krause E Zur Fibrinogenolyse im Nabelvenen und Erwachsenenblut Klin Wschr 41 1085 1963
34 Kunzer W & Markel A Der Proaktivator des fibrinolytischen Systems im Nabelvenen und Sauglingsblut Ann Paediat 202 278 1964
35 Larnen, M J Soulier J P & Minkowski A Le sang du cordon ombilical Etudes Néonatales 1 1 1952
36 Laurell C B Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies Anal Biochem 15 45 1966
37 Markarian M Githens J H Jackson J J Bannon A F Lindley A Rosenblut E Martorell R & Lubchenco L O Fibrinolytic activity in premature infants Relationship of the enzyme system to the respiratory distress syndrome Am J Dis Child 113 31 1967
38 Nilehn J E Separation and estimation of split products of fibrinogen and fibrin in human serum Thrombos Diathes Haemorrh 18 3 1967
39 Nilsson I M Blomback M & von Francken, I On an inherited autosomal haemorrhagic diathesis with antihemophilic globulin (AHG) deficiency and prolonged bleeding time Acta Med Scand 159 35 1957
40 a Nilsson I M & Olow B Determination of fibrinogen and fibrinogenolytic activity Thrombos Diathes Haemorrh 8 297 1962
40 b — Fibrinolysis induced by streptokinase in man Acta Chir Scand 123 247 1962
41 Ochme J Über die Alterabhängigkeit von Retraktion und Profibrinolysin Anderer il Prax 77 487 1959
42 Oski F A & Naiman J L Hematologic problems in the newborn Saunders Philadelphia and London 1966 p 10
43 Paraskevas M Nilsson I M & Martinsson G A method for determining serum inhibitors of plasminogen activation Scand J Clin Lab Invest 14 138 196
44 Phillips L L & Skrodelis V A comparison of the fibrinolytic enzyme system in maternal and umbilical-cord blood Pediatrics 22 715 1958
45 Quie P G & Wannamaker L W The plasminogen–plasmin system of newborn infants Am J Dis Child 100 836 1960
46 Runge H Hartert I & Escher W Vergleichende Untersuchungen der Blutgerinnung im mutterlichen und kindlichen Blut bei noch pulsierender Nabelschnur Gynaecologia 138 337 1954
47 Salazar de Sousa C Les alterations de la coagulabilité sanguine chez le nouveau ne Pédiatrie 9 787 1954
48 — Clotting factors in newborn Etudes Néonatales 5 97 1956
49 Samartzis E A & Cook C D The relationship between age and fibrinolytic activity of serum Acta Paediat Scand 49 724 1960
50 Shamash Y & Rimon A The plasmin inhibitors of plasma A method for their estimation Thrombos Diathes Haemorrh 12 119 1964
51 Smith C A The physiology of the newborn infant Thomas Springfield Ill 1959 3rd ed p 266
52 Stiehm E R Split products of fibrinogen in cord serum [Abst] Pediat Res 1 221 1967
53 Stroder J & Kunzer W Gerinnungsstudien bei Kindern 4 Mitteilung Fibrinogen und Fibrinolyse im Nabelschnurblut Ann Paediat 188 207 1957
54 Taylor P M Concentration of fibrinogen in the plasma of normal newborn infants Pediatrics 19 233 1957
55 Townsend P M The determination of factors in fibrinolysis in umbilical venous blood Ann Paediat 192 133 1959
56 Truelove S C The lability of human fibrinolysin Clin Sci 12 75 1953
57 Usher R Shephard M & Lind J The blood volume of the newborn infant and placental transfusion Acta Paediat Scand 52 497 1963
58 Zilliacus H Ottelin A M & Mattson T Blood clotting and fibrinolysis in human foetuses Biol Neonat 10 108 1966

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level was decreased in accordance with previous investigations The high activator activity is counterbalanced by adequate inhibitory protection with an increase of the α macroglobulin and inhibitors of plasminogen activation and the adult content of antiplasmin The mean fibrinogen values in the *first 48 hours* of life are in the lower part of the normal range but increase on the third day to a *significantly* higher level

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REFERENCES

1 *Albrechtsen O K Fibrinolytic activity in the organism Acta Physiol Scand 47* Suppl 165 *1959*

2 Ambrus C M Weintraub D H Dunphy D Dowd J E Pickren J W Niswander K R & Ambrus J L Studies on hyaline membrane disease I The fibrinolysin system in pathogenesis and therapy *Pediatrics 32* 10 1963

3 Ambrus C M Weintraub D H Niswander K R & Ambrus J L Studies on hyaline membrane disease II The ontogeny of the fibrinolysin system *Pediatrics 35* 91 1965

4 Andersson L Nilsson I M & Olow B Fibrinolytic activity in man during surgery *Thromb Diathes Haemorrh 7* 391 1962

5 Anistowicz Z Euglobulin fibrinolysis in newborn infants healthy prematures and prematures with respiratory distress syndrome (R D S) *Ann Paediat 206* 265 1966

6 Astrup T Tissue activators of plasminogen *Fed Proc* 25 42 1966

7 Beller F K *Die Gerinnungsverhaltnisse bei der Schwangeren und beim Neugeborenen* Barth Leipzig 1957

8 Beller F K Douglas G W & Epstein M D The fibrinolytic enzyme system in the newborn *Am J Obstet Gynec 96 977 1966*

9 Beller F K Goesmer W & Herrschlein H J Tissue activator of the fibrinolytic system in placental tissue *Obstet Gynecol* 20 117 1962

10 Berglund G The fibrinolytic activity in the newborn *Acta Paediat Scand 47* 511 1958

11 Bernardi M Grasso E Nasso S Verga A & Albanesi R Sulla patogenesi dello emorragie del neonato *Il Lattante 24* 219 1953

12 Bonifaci E Baggio P & Gravina E Demonstration of split products of *fibrinogen in the* blood of normal newborns *Biol Neonat 12* 29 1968

13 Bruster H & Pfitzner W Untersuchungen uber das fibrinolytische Potential bei reifgeborenen und jungen Sauglingen *Mschr Kinderheilk 113* 476 1965

14 Chaplin H Observations on fibrinolysis in umbilical cord blood *Clin Sci 13* 111 1954

15 Chu J Clements J A Cotton E K Klaus M H Sweet A Y & Tooley W H Neonatal pulmonary ischemia Part I Clinical and physiological studies *Pediatrics 40* 709 1967

16 Ciulli U & Lurasch C L attivita fibrinolitica del siero di sangue del funicolo ombelicale *Ann Obstet* Ginec 76 207 1954

17 Cope I & Mitchell P The plasminogen-plasmin content of umbilical cord blood *Aust N Z J Obst Gynaec 4* 117 1964

18 *Cope I & Simmons E The fibrinolytic activity* of umbilical cord blood *J Obst & Gynaec Brit Emp 65* 416 1958

19 Ekelund H Hedner U & Astedt B Fibrinolysis in the foetus To be published

20 Engstrom L & Kager L Changes in plasma fibrinolytic activity of newborn infants during first hour after birth *Acta Paediat Scand* 53 326 1964

21 — Fibrinolytic activity in plasma of newborn infants *Acta Paediat Scand 53* 329 1964

22 Fearnley G R Revill R & Moore Tweed J Observations on the inactivation of fibrinolytic activity in shed blood *Clin Sci 11* 309 1952

23 Fisher Sh Schwartz M Gottlieb A Ben Yoseph N & Shapiro S Fibrinolysis fibrinogen and factor XIII in newborn infants *Thrombos Diathes Haemorrh* 20 542 1968

24 Gaburro D Panizza G F & Bonifaci E *Les alterations du systeme fibrinolytique chez le* nouveau ne *Ann de Pediatrie* 38 621 1962

25 Ganrot P O Determination of α_2-macroglobulin as trypsin protein esterase *Clin Chim Acta 14* 493 1966

26 Ganrot P O & Scherstén B Serum α_2-macroglobulin concentration and its variation with age and sex *Clin Chim Acta 15* 113 1967

27 Ganrot P O & Niléhn J E Immunochemical determination of human plasminogen *Clin Chim Acta* 22 335 1968

28 Grossman B J Heyn R M & Rozenfeld I H Coagulation studies in the newborn infant I *Normal infants Pediatrics* 9 182 1952

29 Hathaway W E Mull M M & Pechet G S Disseminated intravascular coagulation in the newborn *Pediatrics 43* 233 1969

30 Howell W L Fibrinolytic activity of the umbilical cord blood *Med Ann D C* 29 491 1960

31 Jenny J & Duckert F Die Gerinnungsverhaltnisse beim lebensfrischen, nichtasphyktischen Neugeborenen nach normaler Geburt und Schwangerschaft *Gynaecologia* *155* 125 1963

32 Karitzky D Pringsheim W Zimmer O & Künzer W Veränderungen der Fibrinogenkonzentration im Plasma gesunder Frühgeborener am 1 Lebenstag *Z Kinderheilk* *105* 73 1969

33 Künzer W & Krause E Zur Fibrinogenolyse im Nabelvenen und Erwachsenenblut *Klin Wschr* 41 1085 1963

34 Künzer W & Merkel A Der Proaktivator des fibrinolytischen Systems im Nabelvenen und Säuglingsblut *Ann Paediat* *202* 278 1964

35 Larrieu M J Soulier J P & Minkowski A Le sang du cordon ombilical *Etudes Neonatales* *1* 1 195

36 Laurell C B Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies *Anal Biochem* *15* 45 1966

37 Markarian M Githens J H Jackson J J Bannon A E Lindley A Rosenblut, E, Martorell R & Lubchenco L O Fibrinolytic activity in premature infants Relationship of the enzyme system to the respiratory distress syndrome *Am J Dis Child* *113* 312 1967

38 Niléhn J E Separation and estimation of split products of fibrinogen and fibrin in human serum *Thromb Diathes Haemorrh* *18* *3* 1967

39 Nilsson I M Blombäck M & von Francken I On an inherited autosomal haemorrhagic diathesis with antihemophilic globulin (AHG) deficiency and prolonged bleeding time *Acta Med Scand* *159* 35 1957

40 *a* Nilsson I M & Olow B Determination of fibrinogen and fibrinogenolytic activity *Thromb Diathes Haemorrh* *8* 297 1962

40 *b* — Fibrinolysis induced by streptokinase in man *Acta Chir Scand* *123* 247 1962

41 Oehme J Über die Altersabhängigkeit von Retraktion und Profibrinolysin *Kinderärztl Prax* 27 482 1959

42 Oski F A & Naiman J L *Hematologic problems in the newborn* Saunders Philadelphia and London 1966 p 10

43 Paraskevas M Nilsson I M & Martinsson, G A method for determining serum inhibitors of plasminogen activation *Scand J Clin Lab Invest* *14* 138 1962

44 Phillips L L & Skrodelis V A comparison of the fibrinolytic enzyme system in maternal and umbilical-cord blood *Pediatrics* 22 715 1958

45 Quie P G & Wannamaker L W The plasminogen-plasmin system of newborn infants *Am J Dis Child* *100* 836 1960

46 Runge H Hartert I & Eicher W Vergleichende Untersuchungen der Blutgerinnung im mütterlichen und kindlichen Blut bei noch pulsierender Nabelschnur *Gynaecologia* *138* 337 1954

47 Salazar de Sousa C Les altérations de la coagulabilité sanguine chez le nouveau-né *Pédiatrie* 9 787 1954

48 — Clotting factors in newborn *Etudes Néonatales* 5 97 1956

49 Samartzis E A & Cook C D The relationship between age and fibrinolytic activity of serum *Acta Paediat Scand* *49* 724 1960

50 Shamash Y & Rimon, A The plasmin inhibitors of plasma. A method for their estimation. *Thromb Diathes Haemorrh* *12* 119 1964

51 Smith C A *The physiology of the newborn infant* Thomas, Springfield Ill 1959 3rd ed p 266

52 Stiehm E R Split products of fibrinogen in cord serum [Abst] *Pediat Res* *1* 221 1967

53 Ströder J & Künzer W Gerinnungsstudien bei Kindern 4 Mitteilung Fibrinogen und Fibrinolyse im Nabelschnurblut *Ann Paediat* *188* 207 1957

54 Taylor P M Concentration of fibrinogen in the plasma of normal newborn infants *Pediatrics* *19* 233 1957

55 Townsend P M The determination of factors in fibrinolysis in umbilical venous blood *Ann Paediat* *192* 155 1959

56 Truelove S C The lability of human fibrinolysin *Clin Sci* *12* 75 1953

57 Usher R Shephard M & Lind J The blood volume of the newborn infant and placental transfusion. *Acta Paediat Scand* 52 497 1963

58 Zilliacus, H Ottelin A M & Mattson T Blood clotting and fibrinolysis in human foetuses *Biol Neonat* *10* 108 1966

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THE RESULTS OF IMMUNOSUPPRESSIVE THERAPY IN CHRONIC RENAL DISEASES IN CHILDREN AND THE ASSESSMENT OF ERYTHROBLASTS BEHAVIOUR

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Immunosuppressive therapy (1 5 11 13 14 17 18) has been employed in forms of chronic renal diseases in children in which other therapeutic methods including corticosteroids had proved ineffective

MATERIAL AND METHODS

6 Mercaptopurine was used in doses of 2 5 mg/kg/48 hr for periods of 3 weeks This therapy was usually associated with 0 5 mg/kg/24 hr of Encorton

Immunosuppressive therapy was administered to 33 children aged 2–17 years (18 boys and 15 girls) There were 9 cases of primary nephrotic syndrome 8 cases in the course of chronic glomerulonephritis 12 with *chronic glomerulonephritis* and 4 with pyelonephritis The diagnosis was established mainly on the basis of clinical criteria and on long term supervision In no case was renal biopsy performed

Renal and liver function tests and peripheral blood counts were carried out in all the children before the beginning of therapy and 1–3 days after it finished

In 18 children aspiration myelograms and careful repeated examinations of peripheral blood were done Nine of these 18 had primary or secondary nephrotic syndrome (group A) and nine had chronic glomerulo or pyelonephritis (group B)

Effectiveness of the therapy was assessed by a scoring system assessing the regression of the symptoms and signs characteristic of each group Improvement scored 80–100 was regarded as complete remission 40–70 as partial remission and cases with scores of less than 40 were considered unimproved

In primary nephrotic syndrome the total protein level albumin α_2-globulin and cholesterol in the plasma were measured as was proteinuria Return of the above parameters to their normal values was scored 20 partial normalization 10 and no effect 0 The sum of scores represented the percentage of complete remission

For example if a complete normalization of protein plasma level albumin and α_2-globulin was obtained—the score was 60 Partial normalization of cholesterol was scored at 10 points and reduction of proteinuria another 10 points In such a case the percentage of complete remission after therapy was 80

In the secondary nephrotic syndrome erythrocyturia was used instead of α_2-globulin

In chronic glomerulonephritis the degree of erythrocyturia in relation to therapy was assessed Complete disappearance of erythrocyturia was taken as 100 90 stood for the persistance of microhematuria (less than 10 million of erythrocytes in 12 hours urine) 60 indicated the reducing of macrohematuria to about one half of the initial number (macrohematuria means more than 20 million of erythrocytes in the urine) 0 signified lack of effect

In pyelonephritis the leucocytes erythrocytes and bacteria counts were carried out but no effect after immunosuppressive therapy was noticed

RESULTS

Using this scoring system the summary review of the effects of immunosuppressive therapy in the presented clinical material is shown in Table 1

Out of 9 children with primary nephrotic syndrome only two did not improve during the period of immunosuppressive therapy

Of these two a girl K E aged 3 years had been born from a third pregnancy during which the mother had been treated with Dara-

Table 1 *Collective results of treatment*

Diagnosis	No of cases	No effect	Partial remission	Complete remission
Primary nephrotic syndrome	9	2	1	6
Secondary nephrotic syndrome	8	2	5	1
Glomerulonephritis	12	1	5	6
Pyelonephritis	4	3	—	1
Total	33	8	11	14
Percentage	100	24.3	33.3	42.4

prum because of toxoplasmosis Two younger children had died immediately after birth Illness had begun a year earlier and despite hospital treatment remission was not achieved During the child's clinical stay her condition continuously deteriorated She died after having varicella and pneumonia

A boy S W 2 years was resistant to corticosteroids from the beginning of the illness Accompanying pneumonia influenced prognosis adversely Autopsy revealed membranous glomerulonephritis and pneumocystosis

As can be seen in Table 1 the results obtained in the secondary nephrotic syndrome were less impressive It is worth noting on the other hand that the remissions persisted and in two children further improvement was observed without additional therapy In three children with partial remissions 6 MP therapy was repeated resulting in a complete remission in one case

In the group of children with chronic glomerulonephritis complete or partial remission were achieved in all but one case a boy

Table 2 *The effect of immunosuppressive therapy on bone marrow results of treatment and toxic manifestations*

Patients (initials)	Age (years)	Sex	Erythroblasts Before treatm	Erythroblasts After treatm	G/E ratio Before treatm	G/E ratio After treatm		Results of treatment No effect	Results of treatment Part remiss	Results of treatment Compl remiss	Toxic manifestations None	Toxic manifestations Blood	Toxic manifestations Liver
Group A													
K A	3		17.7	10.8	3.33	4.27							
D T	11	♂	6.6	12.9	9.31	3.81				+	+		
P B	3		5.8	16.4	12.00	2.60				+	+		
R J	9	♂	11.2	7.0	5.32	1.55				+	+		
N M	4	♀	7.5	18.0	8.44	2.66				+	+		
S W	13	♂	5.4	18.3	11.83	3.06				+	+		
W B	7		11.1	17.3	5.43	3.82			+		+		
D H	9	♂	3.0	12.9	25.00	5.14			+				+
P K	16		7.8	16.5	6.46	3.34		+				+	+
										+	+		
Arithmetic mean			8.4	13.9	9.68	4.13	Total	1	2	6	7	1	2
Group B													
N R	5	♂	25.5	16.4	1.75	3.09							
W Z	10		25.8	19.5	1.74	2.40			+		+		
M L	9		17.4	21.3	3.70	2.97			+		+		
Sz R	13	♂	9.9	20.1	6.78	2.26				+	+		
K Z	15	♀	28.0	—	1.60	—			+		+		
Ch M	14	♂	21.0	19.8	2.48	2.69			+				
P B	11		19.2	11.7	2.32	4.33			+		+		+
K An	8	♀	23.4	20.7	1.78	2.23		+			+		
C Z	11	♀	10.8	18.6	4.75	3.29		+				+	+
								+			+		
Arithmetic mean			20.1	18.5	2.98	2.90	Total	3	5	1		1	2

with nephropathy in the course of Schonlein Henoch disease

Poorest therapeutic results were obtained in the small group of children suffering from pyelonephritis

The influence of immunosuppressive therapy on some bone marrow elements is illustrated in Table 2

In group A percentages of erythroblasts in the bone marrow before treatment were low exceeding 12% in only one case After treatment, a 2–4 fold increase in the percentage of erythroblasts present occurred in six cases

The G/E ratio of erythroblasts in group A show a different range of values before and after treatment increasing only in two cases For the graphic presentation the method of ogives i e of cumulative frequencies curves was used (Fig 1)

The rise in the percentage of erythroblasts was due mainly to an increase in the relative numbers of basophilic and polychromatic erythroblasts while the percentage of eosinophilic erythroblasts remained unchanged

Changes in the granulocytic system under the influence of the immunosuppressive therapy (Table 2) consisted mainly in a slight drop of their percentages but all the values lie within normal limits The ratio of granulocytes to

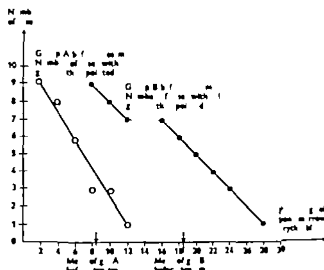


Fig 2 Cumulative curves of bone marrow erythroblasts in group A and B before immunosuppressive therapy

erythroblasts which had been elevated before treatment, tended to become normal after treatment in nearly all cases

As compared to group A the percentages of erythroblasts in group B were higher before treatment (Fig 2)

Under the influence of immunosuppressive therapy the percentages of erythroblasts in the bone marrow in group B behaved differently A distinct rise occurred in two cases in which erythroblasts percentages were low before therapy (Sz R 11 years and C Z 11 years)

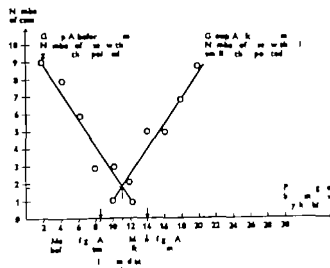


Fig 1 Cumulative curves of bone marrow erythroblasts in group A before and after immunosuppressive therapy

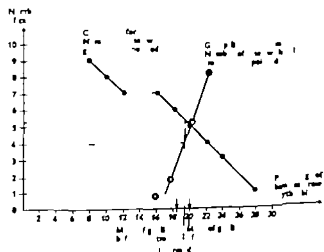


Fig 3 Cumulative curves of bone marrow erythroblasts in group B before and after immunosuppressive therapy

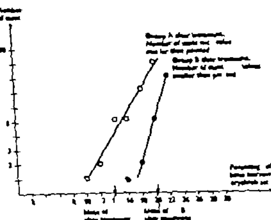


Fig 4 Cumulative curves of bone marrow erythroblasts in Group A and B after immunosuppressive therapy

It can be seen in Fig 3 that the percentages of erythroblasts in group B before and after treatment were similar

D spite of the increase in erythroblasts in the bone marrow after therapy in group A their values remained lower than in group B (Fig 4)

The granulocytes in patients of group B were affected little by the treatment In all cases the bone marrow was moderately or richly cellular

Hemoglobin levels and red blood cell counts were similar in both groups of children Moderate normochromic anemia was observed

In group A granulocytes counts which were elevated before treatment (mean 8200/mm³) dropped after treatment in all cases except one but neutropenia was not observed (mean 5700/mm³) Mean numbers of granulocytes in this group were higher than in group B even after treatment In group B granulocyte counts were normal with a tendency to decrease after treatment

Blood platelet counts in both groups were within normal limits with a slight tendency to decrease after treatment

Reticulocyte counts performed in most cases ranged between 0 3-0 9% In group A a slight increase was observed after treatment and in group B there was no change

DISCUSSION

Immunosuppressive therapy gave encouraging results in children who failed to improve after corticosteroid therapy and in whom prognosis was poor

The therapeutic results observed in the primary nephrotic syndrome are in agreement with the findings of other authors (7 12 15) The more favorable results obtained for chronic glomerulonephritis were probably due to shorter duration of the disease process in our patients Our observations indicate that immunosuppressant therapy should start with low doses of 6 MP (2 5 mg/kg/48 hr) which exert beneficial effects with almost no toxicity We cannot agree with Shearn (16) and Grupe (8) who administered high daily doses of 6 MP provoking toxic symptoms in all cases Harmful effects of 6 MP on the peripheral blood and liver were observed in only 2 of our patients both of whom failed to improve after immunosuppressive therapy This observation seems to correspond to the observations of Halikowski *et al* (10) who noted that in acute leukemia children in whom drugs elicited toxic symptoms were usually refractory to treatment

Hematologic studies in our patients confirmed the phenomenon (2 3 4 6) of a lack of correlation between percentages of erythroblasts in the bone marrow and the degree of anemia Similar degrees of anemia were observed in children with low numbers of erythroblasts in the bone marrow (group A) and in children with normal erythroblast percentages (group B)

In children with the nephrotic syndrome low percentages of erythroblasts in the bone marrow increased after immunosuppressive therapy but anemia has not improved

On the basis of the observed facts the hypothesis may be proposed that at least two factors influence the erythropoietic system in the bone marrow Presumably in the nephrotic syndrome there is a deficiency of erythropoietin I which stimulates multiplication of erythroblasts in the bone marrow while activity of erythropoietin II which regulates maturation

of erythroblasts, production of erythrocytes and synthesis of hemoglobin, is normal This may be suspected on the basis of the low percentages of erythroblasts in the bone marrow and only moderate anemia In glomerulonephritis the opposite was observed anemia of the same degree was accompanied by normal numbers of erythroblasts in the bone marrow suggesting deficiency of erythropoietin II but normal or enhanced activity of erythropoietin I

At present the influence of immunosuppressive therapy on bone marrow erythroblasts is difficult to explain

SUMMARY

Immunosuppressive therapy (6 Mercaptopurine) has been used in 33 children with chronic renal diseases resistant to other therapeutic methods Peripheral blood counts were done in all the children and in 18 of them bone marrow examinations were performed

The results of immunosuppressive therapy were encouraging many complete and partial remissions being obtained

Children with nephrotic syndrome showed low percentages of erythroblasts in bone marrow This was not observed in children suffering from chronic glomerulo and pyelonephritis Immunosuppressive therapy provoked increase of erythroblasts in bone marrow of children with nephrotic syndrome

REFERENCES

1 Adams D A Gordon A & Maxwell M H Azathioprine treatment of immunological renal disease *JAMA 199* 459 1967
2 Aleksejew G I The typical reactions of bone marrow in renal diseases The mechanism of destroying bone marrow production (In Russian) *Ter Arkh 22* 58 1950
3 Bock H E Nieth H & Solth K Anamie bei Niereninsuffizienz *Deutsch Med Wschr 87* 573 1962
4 Callen I R & Limarzi L R Blood and bone marrow studies in renal disease *Amer J Clin Path 20* 3 1950
5 Chaptal J Jean R Bonnet H Navarro M & Leoret R Intéret du chloraminophène dans le traitement de certains syndromes nephrotiques *Arch Franc Pediat 22* 473 1965
6 Choremis C Megas H Liaromati A & Michael S Bone marrow studies in acute glomerulonephritis *Helv Paediat Acta* 17 138 1962
7 Farnaux J P Le traitement du syndrome nephrotique idiopatique Règles générales *La Medecine Infantile 74* 655 1967
8 Grupe W F & Heymann W Cytotoxic drugs in steroid resistant renal disease *Amer J Dis Child 112* 448 1966
9 Halikowski B Kucharska K & Sancewicz Pach K Clinical results of immunosuppressive therapy in nephrotic syndrome and nephritis in children (In Polish) *Pediat Pol* In press
10 Halikowski B Armata J Garwicz S Wyszkowski J & Cyklis R Drug sensitivity and resistance in acute leukemia in children and toxic action of the applied drugs (In Polish) *Pediat Pol 43* 669 1968
11 Japa J Zinger Z Dzierza I & Rozek K Up to date results of treatment with cyclophosphamide (endoxan) (In Polish) *Pol Arch Med Wewnet 38* 431 1967
12 Lagrue G Bariety J Halpern B & Milliez P Les immuno dépresseurs en nephrologie-essais de traitement des glomerulopathies chroniques primitives *Presse Med 75* 1779 1967
13 Lagrue G Bariety J Canlorbe P Vassal J & Milliez P La chimiotherapie dite immuno depressive dans les syndromes nephrotiques primitifs de l enfant *Presse Med 75* 1773 1967
14 Perlinska Sznejder L & Gutka A Nitrogranulogen therapy in nephrotic syndrome (In Polish) *Pol Tyg Lek 20* 144 1965
15 Saxena K M & Crawford J D The treatment of nephrosis *New Engl J Med 272* 522 1965
16 Shearn M A Mercaptopurine in the treatment of steroid resistant nephrotic syndrome *New Engl J Med 273* 943 1966
17 West C D Hong R & Holland N H Effect of cyclophosphamide on lipoid nephrosis in the human and aminonucleoside nephrosis in the rat *J Pediat 68* 516 1966
18 West C Use of combined hormone and mechlorethamine (Nitrogen Mustard) therapy in lipoid nephrosis *Amer J Dis Child 95* 498 1958

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HEMOGLOBIN HEMATOCRIT AND RED BLOOD CELL COUNT IN CAPILLARY (SKIN PRICK) BLOOD COMPARED TO VENOUS BLOOD IN CHILDREN

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Usually no distinction is made in the literature between figures for hemoglobin (Hb) hematocrit (Htc) and red blood cell count (RBC) in capillary and venous blood in children. Most textbooks for instance present data for normal Hb Htc and RBC without reference to the type of blood studied. Some of the data for normal blood values in infancy and childhood are actually from venous blood studies (2 5 6). In newborn infants skin prick samples from the heel have higher Hb than samples taken simultaneously from vein (8). It seems important to know whether there also in children may be a regular difference between venous Hb Htc and RBC and the corresponding figures in capillary (skin prick) blood obtained with a standardized technique used in clinical practice.

MATERIAL AND METHODS

The material consists of 30 children aged 2 to 13 years admitted to the Children's Hospital. Blood studies were also performed on 30 adult (15 males and 15 females) members of the staff at the hospital. Capillary blood was obtained from a 3 mm deep puncture in the finger with a "Medipoint" blood lancet. The first drop or two was discarded. Slight pressure had usually to be made some distance from the skin prick. None of the studied subjects had cyanotic or cold fingers and it was therefore not considered necessary to immerse the finger in warm water before the puncture. Venous blood samples were obtained from an ante-cubital vein; the external jugular vein was used in one child. The application of a tourniquet was found necessary in order to obtain the samples from the antecubital vein.

Hb Htc and RBC were determined in duplicate by methods reported previously (7).

RESULTS

Higher Hb Htc and RBC were found in venous blood in children as well as in adults (Table 1). The difference seems to be most marked in children with a mean difference of Hb of 0.5 g/100 ml a mean difference of Htc of 1.7 vol per cent and a mean difference of RBC of 0.18 mill/mm³. In adults there was only a difference in RBC of 0.08 (males) and 0.10 (females) mill/mm³.

In children higher venous Hb was found in 26 of the 30 cases, higher venous Htc in 21 of 28 cases and higher RBC in 25 of 29 studied cases.

The differences between Hb Htc and RBC in venous versus capillary blood were all significant at the 0.1 per cent level in children. The difference between Htc in venous versus capillary blood was significant at the 0.1 per cent level in adult females and between Hb at the 0.5 per cent level in both adult males and females while no significant difference was found between Htc in adult males or between RBC in both groups of adults.

DISCUSSION

This study shows that a significant difference may exist in children between Hb Htc and

Table 1 *Comparison between venous and capillary blood values*

	Hemoglobin		Hematocrit		Red blood cells	
	No of cases	Mean (g/100 ml)	No of cases	Mean (vol)	No of cases	Mean (mill/mm³)
Children						
Venous	30	12 63	28	39 1	29	4 51
Capillary	30	12 13	28	37 4	29	4 33
Difference		0 50		1 7		0 18
Adult males						
Venous	15	14 78	14	45 7	12	4 79
Capillary	15	14 43	14	44 7	12	4 71
Difference		0 35		1 0		0 08
Adult females						
Venous	15	13 19	15	41 8	14	4 33
Capillary	15	12 89	15	40 2	14	4 23
Difference		0 30		1 6		0 10

RBC in venous blood and blood from a finger prick The observed difference in the means *in the children about 4 per cent of the venous* blood values are too high to be ignored It seems also important to be aware of the possible error introduced by using the results of venous and capillary blood determinations interchangeably in a child A difference between capillary and venous Hb of 1 3 g/100 ml Htc of 3 5 vol per cent and RBC of 0 38 mill/mm³ was found in the same child

The method of using venous blood for determining Hb Htc and RBC is according to Wintrobe (9) definitely more accurate than the use of capillary blood Care must be taken however to avoid any error introduced by congestion of the arm This may be difficult to avoid in a child Furthermore it is out of the question to perform Hb determination on venous blood as a routine procedure even in children who are inpatients

It is not quite clear whether Hb Htc and RBC of venous and capillary blood are the same (3) Studies by Gibson actually indicate that Hb Htc and RBC of true capillary blood may be significantly less than those of venous blood

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SUMMARY

Hb Htc and RBC in capillary (skin prick) and venous blood have been compared in 30 children and in 30 adults (15 males and 15 females) Capillary blood was obtained from a 3 mm deep puncture in the finger with a Medipoint blood lancet Higher mean values were found in venous blood particularly in the children

REFERENCES

1 Andresen M I & Mugrage E R Venous and peripheral blood cell values *Amer J Clin Path 8* 46 1938
2 Baar H S Baar S Rogers K B & Stransky

R R. *Disorders of blood forming organs in childhood* S Karger Basel and New York 1963
Dacie J & Lewis S M. *Practical Haematology* J & A Churchill Ltd London 1963 3rd edition p 10
Gibson J G Seligman A. M Peacock W C Aub J C Fine J & Evans R D The distribution of red blood cells and plasma in large and minute vessels of the normal dog determined by radioactive isotope of iron and iodine *J Clin Invest* 25 848 1946
Guest G M Brown E W & Lahey M E Normal blood values in infancy and childhood *Pediat Clin N Amer* 4 337 1957
Miale J B *Laboratory Medicine Hematology* The C V Mosby Company ST Louis 1967 3rd edition
Moe P J Normal red blood picture during the first three years of life *Acta Paediat Scand* 54 69 1965
8 Mollison P L *Blood transfusion in clinical practice* The C V Mosby Company St Louis 1967 3rd edition p 579
9 Wintrobe M M *Clinical hematology* H Kimpton London 1967 6th edition p 411

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Table 1 *Comparison between venous and "capillary blood values*

	Hemoglobin		Hematocrit		Red blood cells	
	No of cases	Mean (g/100 ml)	No of cases	Mean (vol)	No of cases	Mean (mill/mm³)
Children						
Venous	30	12 63	28	39 1	29	4 51
Capillary	30	12 13	28	37 4	29	4 33
Difference		0 50		*1 7*		*0 18*
Adult males						
Venous	15	14 78	14	45 7	12	4 79
Capillary	15	14 43	14	44 7	12	4 71
Difference		0 35		1 0		*0 08*
Adult females						
Venous	15	13 19	15	41 8	14	4 33
Capillary	15	12 89	15	40 2	14	4 23
Difference		0 30		1 6		0 10

RBC in venous blood and blood from a finger prick The observed difference in the means in the children about 4 per cent of the venous blood values are too high to be ignored It seems also important to be aware of the possible error introduced by using the results of venous and capillary blood determinations interchangeably in a child A difference between capillary and venous Hb of 1 3 g/100 ml Htc of 3 5 vol per cent and RBC of 0 38 mill/mm³ was found in the same child

The method of using venous blood for determining Hb Htc and RBC is, according to Wintrobe (9) definitely more accurate than the use of capillary blood Care must be taken however to avoid any error introduced by congestion of the arm This may be difficult to avoid in a child Furthermore it is out of the question to perform Hb determination on venous blood as a routine procedure even in children who are inpatients

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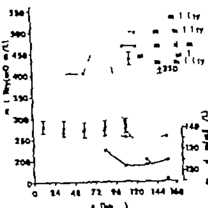


Fig. 1. Serum and urine osmolalities and serum sodium in case 1. Note the hypertonic urine and low serum sodium and osmolality.

were no seizures, hydration appeared normal and there was no edema.

Because of another study then in progress in which serum and urine osmolality were being measured in newborns, serum and urine were collected daily, frozen and stored. Several weeks later osmolality was measured. The results of these sequential determinations are shown in Fig. 1. Although the initial serum values of 266 and 270 mOsm/l on days 2 and 4 were within normal limits, thereafter below normal values (7) of 254 mOsm/l were obtained on each of days 5, 6 and 7. The serum sodium values of 130 mEq/l on day 4 and 120–125 mEq/l on days 5–7 reflect this change.

The patient's urine was more concentrated than that of control babies fed the same formula, rising to 513 mOsm/l on the fourth day. The mean urine osmolality of 21 normally hydrated 4 day old newborns was 118 mOsm/l with a range of 49–363 mOsm/l (7). A blood urea nitrogen (BUN) of 16.5 on the fourth day indicated that dehydration or diminished glomerular filtration rate did not play a role in producing the concentrated urine. McCance & Widdowson have shown that the mean BUN of normal 4 day-old infants fed breast milk was 29.1 mg/100 ml (15).

After the initial asphyxic episode the baby appeared well; he was discharged on the seventh day apparently in good health. At that time the urine had become dilute (122 mOsm/l). No specific therapy had been instituted since the results of the osmolality studies were known only after the baby had been discharged.

Case 2

This 2285 g male infant was the product of a 42 week gestation. Because of prolonged labor, delivery was by Caesarian section. The infant was pale, blue and flaccid at birth. No resuscitation was performed. On the third day dehydration and a temperature of 100.3 F were noted and he was transferred to the Montreal Children's Hospital. On admission the baby appeared dehydrated and fetally malnourished; the weight was 2080 g. The serum sodium was 125 mEq/l, the potassium 6 mEq/l, the calcium 7 mg/100 ml, the glucose 144 mg/100 ml, the BUN 52 mg/100 ml. There was a mild compensated metabolic acidosis. The urine osmolality was 470 mOsm/l. Meningitis due to *E. coli* was diagnosed and antimicrobial therapy was started. A single brief seizure was noted on the day of admission and a bulging fontanel was present for one week. Values for serum sodium, urine osmolality and sodium intake are shown in Fig. 2a. For the first 24 hours after admission the serum sodium remained between 120–125 mEq/l despite intravenous administration of a solution of sodium chloride (50 mEq/l) calculated to give 10–12 mEq/day. On the fourth day of life 12 mEq of hypertonic sodium chloride solution (400 mEq/l) were given over a 4 hour period; this was calculated to elevate the serum sodium to 133 mEq/l and was in addition to the 12 mEq infused over the total 24 hour period. The serum sodium rose transiently to 135 mEq/l; 16 hours later it had fallen to 125 mEq/l.

The combination of persistent hyponatremia, hypertonic urine and normal renal function suggested the possibility of an inappropriate ADH syndrome. Fluids were withheld for an 8 hour period on the fifth day of life and the serum sodium rose to 135 mEq/l. A water loading test, to be described, was done on the sixth day. At that time the BUN was 26 mg/100 ml. On the seventh day the baby developed diarrhea and 10–12 mEq of sodium per day which previously had no effect on the hyponatremia was then sufficient to maintain a normal serum sodium. The urine became dilute on the eighth day of life. The weight varied between 2220 and 2350 g from the fifth to the thirteenth day when a gradual and sustained weight gain was seen.

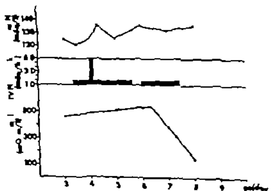


Fig. 2a. Serum sodium, intravenous sodium intake and urine osmolality in case 2. Note the hypertonic urine and transient rise in serum sodium on day 4 after hypertonic sodium infusion.

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HYPONATREMIA FOLLOWING ASPHYXIA NEONATORUM

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Hyponatremia in the newborn may result from *adrenal insufficiency* salt losing virilizing adrenal hyperplasia water intoxication or renal disease Maternal hyponatremia during labor can also lead to hyponatremia in newborns (21)

Schwartz and his associates first recognized the syndrome of inappropriate secretion of antidiuretic hormone (ADH) in hyponatremic adults with bronchogenic carcinoma (20) Briefly this syndrome is characterized by hyponatremia with hypertonic urine in the absence of renal adrenal cardiac or hepatic disease The hyponatremia is not corrected by the *administration of hypertonic saline but is* corrected by fluid restriction

Since Schwartz's description this syndrome has been reported in patients with intracranial disease (6) myxedema (11) acute intermittent porphyria (13) pulmonary tuberculosis (24) and Vincristine therapy (8) McCrory & MacAulay reported a brain damaged 5 month old infant whose hyponatremia was associated with high blood levels of ADH (18) To our knowledge their patient is the youngest described with this syndrome

The purpose of this report is to describe 3 newborn with asphyxia neonatorum whose hyponatremia appears best explained by an inappropriate secretion of ADH

Medical Research Council of Canada Fellow 1966–1967 *Present address* Department of Pediatrics McMaster University Hamilton Ontario

Associate Professor of Pediatrics McGill University and Physician and Director Renal Laboratory Montreal Children's Hospital

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Sterilon Corp Buffalo N Y

Fiske Associates Inc Uxbridge Massachusetts

In our nursery babies are offered 30–45 ml of glucose 5 in water every 4 hours for the first 3 feedings thereafter a *commercial low solute cow's* milk preparation (Enfalac 20 calories/30 ml—Mead Johnson Canada Ltd Toronto Ontario) is given at 4 hourly intervals beginning with about 60 ml/feeding on the second day and increasing to about 100–120 ml by the seventh day

METHODS

Blood was taken by femoral venepuncture in cases 1 and 2 and from a warmed heel in case 3 Urine was collected in disposable plastic containers and samples were analyzed within 2 hours or frozen for later determination In case 3 urine was collected on a metabolic bed Osmolality was measured with a Fiske osmometer* using 0 2 ml samples Reproducibility with this instrument in our laboratory is ± mOsm/l

CASE REPORTS

Case I

This male infant weighing 4182 g at birth was the product of a 40 week gestation Delivery was by Cesarian section because of cephalo pelvic disproportion The condition at birth was poor he was cyanosed flaccid and required intubation The Apgar score at one minute was 2 Shortly after intubation and manual positive pressure oxygen therapy the colour and tone improved Thereafter the *infant* appeared well and took oral feedings adequately There

Blood gases from a warmed heel at one hour of age showed a severe combined respiratory and metabolic acidosis pH 7 135 PCO 50 mm Hg base excess −12 1 mEq/l and PO in room air was 35 mm Hg Blood sugar prior to oral or intravenous feedings was 180 mg/100 ml Intramuscular chloromycetin was begun at 25 mg/kg/day Chest X ray showed moderate cardiomegaly with severe vascular congestion Electrocardiogram showed a right axis deviation At 3 hours of age an intravenous drip of 10% glucose and water was begun at 65 ml/kg/day containing 15 mEq/day of sodium bicarbonate The liver was 5 cm below the costal margin At 7 hours of age the child was put in 50% oxygen and was digitalized because of persistent cardiomegaly and pulmonary vascular congestion He was given an additional 7 5 mEq of sodium bicarbonate intravenously over 2 hours and was started on hydrocortisone (7 5 mg every 4 hours for 6 doses) At 28 hours the infant had a generalized seizure The fontanel was not bulging The blood sugar was 229 mg/100 ml At 24 hours of age the liver was 2 cm below the costal margin and the child was no longer in heart failure and his colour and tone were much improved.

At 37 hours the patient was lethargic and edematous The weight had increased from 2940 g at birth to 3050 g the serum sodium had fallen from 135 mEq/l at 12 hours of age to 128 mEq/l and urine output was negligible A diagnosis of inappropriate ADH response was entertained a repeat serum sodium at 41 hours was 124 mEq/l with a serum osmolality of 252 mOsm/l Simultaneous urine osmolality was 359 mOsm/l The urine sodium on this sample was 4 5 mEq/l and urine sodium determinations during the following 2 days ranged from 0 5 mEq/l to 13 5 mEq/l the latter following an infusion of hypertonic saline Non protein nitrogen was 75 mg/100 ml

Since the child had continuing central nervous system signs it was elected to stop intravenous fluids and attempt to induce a diuresis with an osmotic agent Oral glycerol was given (1 g/kilo) by nasogastric tube (7) Four hours later the patient had voided only 10 ml of hyperosmolar (525 mOsm/l) urine The serum sodium had dropped further to 170 mEq l The serum osmolality however had risen from 252 to 277 mOsm l The patient appeared more alert By hours following the glycerol the patient had voided 23 ml the serum sodium was 132 mEq/l and the serum osmolality 269 mOsm/l Sixteen hours after glycerol administration the serum sodium had risen to 138 mEq/l with an accompanying serum osmolality of 266 mOsm/l The total diuresis was 194 ml The central nervous system status was markedly improved and the child had lost 40 gm during the 30 hour period following glycerol administration despite an intake of 120 ml of milk The subsequent course was unremarkable The lowest urine osmolality (110 mOsm/l) was recorded on the seventh day Non protein nitrogen at that time was 25 Follow up examination at 4 months revealed a healthy infant

DISCUSSION

The salient features of the syndrome of inappropriate secretion of ADH have been summarized by Haden & Knox (12) They include

1 Hyponatremia of moderate to marked degree without significant renal adrenal hepatic or cardiac disease
2 Excretion of a hypertonic urine
3 Normal or low blood urea nitrogen
4 Urinary loss of administered sodium
5 Inability to maintain normal serum sodium concentrations by infusion of hypertonic saline
6 Correction of hyponatremia by fluid restriction

ADH production is termed inappropriate in this syndrome because the secretion of ADH is normally inhibited by hypotonic plasma This inhibition causes the free water clearance to rise leading to the excretion of a dilute urine thus permitting plasma osmolality ro rise to normal levels The excretion of hypertonic urine in the face of hypotonic plasma and normal renal function implies that there is continuing ADH secretion which is inappropriate for the level of serum osmolality

Cases 1 and 2 manifested most of the features of this syndrome A hypertonic urine was elaborated in the presence of hyponatremia and in the absence of demonstrable renal adrenal hepatic or cardiac disease In case 2 the administration of hypertonic saline produced only a transient elevation of serum sodium Although urine sodium was not measured it is possible that the rapid decline in serum sodium following hypertonic saline infusion was due to urinary loss The urinary loss of administered sodium is considered to be related to a decrease in aldosterone secretion secondary to an expanded extracellular fluid volume (20) Case 3 presents some problems with respect to the diagnosis as defined by Haden & Knox (12) The syndrome as described in adults requires the absence of renal and cardiac dis

Water Load Studies (Fig 2 b)

Weil noted that infants given a water load of 30–50 ml/kg produced a peak diuresis in 50-120 min (23) McCrory found that a normal 5 month old infant diluted his urine one hour after a water load, whereas a patient with high ADH levels did not dilute his urine until 3 hours after the load (18) In the absence of renal, hepatic adrenal or cardiac dysfunction the ability to deliver a water load depends on the ability to suppress ADH secretion If a water load cannot be delivered in a normal manner one can assume an inability to suppress ADH

The following water load study was carried out in case 2 on the sixth and fifteenth days, 2 normal 6 day old infants served as controls Baseline serum and urine were taken for osmolality just prior to a scheduled feeding Thirty millilitres per kilogram of 5% glucose in water was then given by gavage over a 5–10 min period instead of the feeding Osmolality was determined on each urine voided over the next 6 hours and on serum specimens taken at hourly intervals In the controls a fall in serum osmolality of 4 mOsm/l was seen at one hour with a return to pre test levels by 2 hours In the first study in case 2 a slightly greater fall (8 mOsm/l) was seen at one hour and pre test levels had not been reached by 4 hours

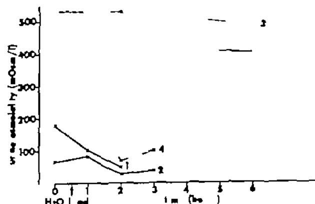


Fig 2 b Changes in urine osmolality after water loading in normal 6 day old infants and in case 2 at age 6 and 15 days Note the decrease in urine osmolality at 2 hours in the controls and in the patient at 15 days Note also the persistently hyperosmolar urine for up to 6 hours in case 2 at age 6 days

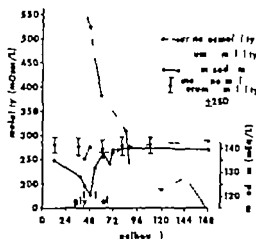


Fig 3 a Case 3 Serum sodium and serum and urine osmolality Note the high urine and low serum osmolality and the fall in serum sodium paralleling the initial increase in serum osmolality

By 2 hours urine osmolality had decreased 50 ₒ in the controls Although a similar response was seen in case 2 at day 15 the urine osmolality remained greater than 500 mOsm/l as late as 6 hours following the water load performed on the sixth day

Case 3 (Fig 3)

This full term 2940 g male infant was born after a 16 hour labor to a gravida 1 mother Membranes were ruptured artificially 6 hours prior to delivery and meconium staining of the amniotic fluid was noted There were no other signs of fetal distress At birth the infant was asphyxiated and meconium stained Positive pressure ventilation was given with bag and mask until spontaneous respirations were sustained at 2 / min The Apgar score at one minute was 3 and at 5 minutes 6 at which time the heart rate was 100 The baby was still cyanotic pale flaccid with a weak cry grunting respirations and marked subcostal retractions

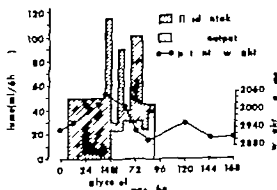


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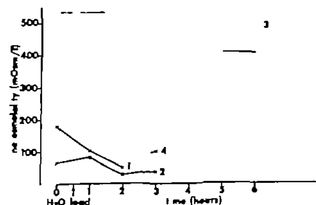


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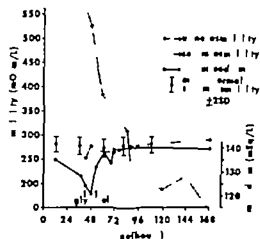


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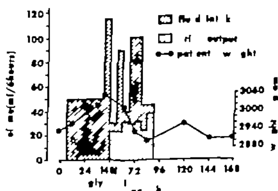


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4 Campan, L. Arbus L. & Lazorthes Y. Note sur le traitment de l'oedeme cerebral par le glycérol. Neurochirurgie II 348 1965
5 Castore G. Gandetio B & Virno M. Oral glycerol for the reduction of intracranial pressure. J Neurosurg 21 278 1964
6 Carter N W. Rector F C & Seldin D W. Hyponatremia in cerebral disease resulting from inappropriate secretion of antidiuretic hormone. New Eng J Med 264 67 1961
7 Feldman W & Drummond K D. Serum and urine osmolality in normal newborns. (In press Canad Med Ass J)
8 Fine R N., Clarke R R & Shore N A. Hyponatremia and Vincristine therapy: syndrome possibly resulting from inappropriate antidiuretic hormone secretion. Amer J Dis Child 112 256 1966
9 Fisher D A. Pyle H R. Porter J C. Beard A G & Panos T C. Control of water balance in the newborn. Amer J Dis Child 106 137 1963
10 Goldberg M. Hyponatremia and the inappropriate secretion of antidiuretic hormone (Editorial) Amer J Med 35 293 1963
11 Goldberg M & Reivich M. Studies on the mechanism of hyponatremia and impaired water excretion in myxedema. Ann Intern Med 56 120 1962
12 Haden H T & Knox G W. Cerebral hyponatremia with inappropriate antidiuretic hormone syndrome. Amer J Med Sci 249 381 1965
13 Hellman E S. Tschudy D P & Bartter F C. Abnormal electrolyte and water metabolism in acute intermittent porphyria. Amer J Med 32 734 196
14 Janovsky M. Martinek J & Stanincova, V. Antidiuretic activity in the plasma of human infants after a load of sodium chloride. Acta Paediat Scand 54 543 1965
15 McCance R A & Widdowson E M. Blood urea in the first days of life. Lancet II 797 1947
16 — Metabolism and renal function in the first two days of life. Sympos Quant Biol 19 161 1954
17 — Protein catabolism and renal function in the first two days of life in premature infants and multiple births. Arch Dis Child 30 405 1955
18 McCrory W W & Macaulay D. Idiopathic hyponatremia in an infant with diffuse cerebral damage. Pediatrics 20 23 1957
19 Obes Polleri J. Ensayo de la glicerina en el dano cerebral del recien nacido. Arch Pediat Urug 36 601 1965
20 Schwartz W B. Bennett W. Curelop S & Bartter F C. Syndrome of renal sodium loss and hyponatremia probably resulting from inappropriate secretion of antidiuretic hormone. Amer J Med 23 529 1957
21 Strauss J. Fluid and electrolyte composition of the fetus and the newborn. Pediat Clin N Amer 13 1077 1966
22 Thomson J. Observations on the urine of the newborn infant. Arch Dis Child 19 169 1944
23 Weil W B. The evaluation of renal function in infancy and childhood. Amer J Med Sci 229 678 1955
24 Weiss H & Katz, S. Hyponatremia resulting from apparently inappropriate secretion of antidiuretic hormone in patients with pulmonary tuberculosis. Amer Rev Resp Dis 92 609 1965

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Canada

Key words: Hyponatremia, antidiuretic hormone, asphyxia neonatorum

ease Case 3 had congestive failure secondary to severe asphyxia, although at the time hyponatremia and urine hypertonicity developed the cardiac problem was controlled The presence of cardiomegaly and pulmonary vascular congestion is a major feature of severe asphyxia neonatorum (3) it may thus be impossible to dissociate the role of cardiac failure from that of inappropriate ADH secretion in such infants The slight elevation of non protein nitrogen was probably due to pre renal causes (17)

The use of oral glycerol as an osmotic agent has been previously described (2 4 5 19) In our patient glycerol administration was followed by a substantial diuresis which began 4 hours after the first dose and lasted 24 hours with weight loss despite continued oral intake The initial drop in serum sodium from 124 to 120 mEq/l following glycerol administration in the face of a rising serum osmolality may represent an initial shift of fluid from intracellular to extracellular spaces prior to the onset of diuresis The low urine sodium concentrations remain unexplained

Using a rat bioassay Janovsky and others were unable to detect antidiuretic activity in the plasma of infants under 2 1/ months (14) Ames however was able to detect ADH in the urine of newborns under 3 days of age after fluid deprivation (1) Because bioassay techniques are difficult to perform with reproducibility other workers have used indirect techniques to detect qualitative and quantitative ADH responses These include production of a concentrated urine following hypertonic saline administration or fluid deprivation Fisher using infusion of 5° sodium chloride showed that newborns could produce a concentrated urine (9) Thomson found that during the first 3 days of life infants were able to respond to a limited fluid intake by excreting a concentrated urine (22) The data of these workers may be interpreted as evidence in favour of ADH secretion in newborn infants

The mechanism which leads to the inappropriate secretion of ADH is unknown Most cases reported to date have had lesions of the thorax or central nervous system An irritative lesion involving thoracic volume receptors or central nervous system osmoreceptors has been postulated as a persisting stimulus for ADH secretion (10) The patients in the present report had asphyxia at birth *E coli* meningitis was also present in the second newborn It is considered possible that an insult to the central nervous system in each infant served as a stimulus to ADH secretion The observations of McCance & Widdowson that mature infants born after prolonged delivery or with asphyxia neonatorum may have smaller than normal urine volumes (16) is of interest in this context It is suggested that this condition may be more common than suspected and that serum sodium levels and urine osmolality or specific gravity be assessed in newborns with asphyxia neonatorum or neonatal meningitis Specific therapy for hyponatremia in this condition consists of fluid restriction rather than administration of hypertonic saline Glycerol may be of value in those patients in whom central nervous system signs due to cerebral edema are evident

SUMMARY

Three newborns with transient asphyxia developed hyponatremia and hypertonic urine Inappropriate secretion of ADH is proposed as a reasonable explanation for these findings Serum sodium and urine concentration should be assessed in newborns with asphyxia or meningitis since this condition may be more common than realized

REFERENCES

1 Ames R G Urinary water excretion and neurohypophyseal function in full term and premature infants shortly after birth *Pediatrics 12* 272 1953
2 Buckell M & Walsh L Effect of glycerol by mouth on raised intracranial pressure in man *Lancet II* 1151 1964
3 Burnard E D & James L S Failure of the heart after undue asphyxia at birth *Pediatrics 28* 545 1961

Table 1 *Clinical and haematological data*

Case no	Age (months) and sex	Weight (kg) Degree of malnutrition	Serum proteins (g/100 ml) alb /glob	Haemoglobin (g/100 ml)	Serum iron Serum iron binding Cap (γ/100 ml)
1	30/♂	6 3/IV	7 8 = 5 3/2 5	6 75	—
2	36/♂	6 8/IV	6 3 = 3 8/2 8	6 65	40/333
3	30/♀	6 71/IV	6 5 = 4 4/2 1	8 45	—
4	18/♀	5 5/IV	7.2 = 4 6/2 6	3 0	136/312 5
5	24/♂	5 83/IV	—	6 95	—
6	18/♀	6 38/III	6 4 = 4 2/2 2	8 45	80/390
7	18/♂	6 7/III	4 6 = 1 5/3 1	7 40	—
	27	10 3/I	6 0 = 3 8/2 2	10 80	—
8	18/[illegible]	6 24/III	4 2 = 2 2/2 0	7 17	90/390
	25	10 8/—	6 8 = 4 2/2 6	11 55	—
9	36/♀	8 5/III	6 4 = 4 4/2 0	3 85	28/314
9	42	10/—	5 6 = 3 0/2 6	12 30	41/417
10	9/♂	4 8/III	3 4 = 1 8/1 6	9 20	—
11	10 ♂	5 9/III	6 1 = 3 9/2 2	10 05	—
12	14/♂	5 8/III	—	9 [illegible]0	—
13	14/♂	7 5/II	7 = 5 5/1 5	9 20	67/334
	19 5	10 1/I	—	10 80	—
14	30/♀	10 3/II	4 5 = 2 5/2 0	6 95	64/350
15	12/♂	6 3/II	7 5 = 5 5/2 0	9 50	—
16	30/♀	8 7 II	6 5 = 4 5/2 0	11 55	—
17	24/	7 5/II	6 8 = 4 1/2 7	11 40	—
18	18 ♂	8 1/II	6 8 = 3 8/3 0	12 30	82/390
19	12/	6 5/II	6 6 = 4 2/2 4	9 6	95/346
0	18 ♂	7 8 II	6 8 = 3 8/3 0	11 25	—
21	36/	9 9 II	4 8 = 2 8/2 0	5 45	42/408
	40	11 5/I	6 2 = 4 0/2 2	10 05	—
22	[illegible]0	8 7/II	6.2 = 4 0/2 2	8 30	183/248
23	24 ♂	9 3 I	4 [illegible] = 2 2/2 0	6 95	157/350
	27	13 —	7 0 = 4 5/2 5	11 85	—
4	36 ♂	12/I	6.2 = 3 7/2 5	9 65	49/350

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JEJUNAL MUCOSA IN INFANTILE MALNUTRITION

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Malnutrition is still one of the major problems in developing countries (24) This is also a common condition in Turkey a study carried out at this hospital in 1958 disclosed that 43 per cent of the children under two years of age seen at the out patient clinics had some degree of malnutrition (14)

Although the different aspects of infantile malnutrition have been extensively studied in recent years histological studies of the small intestine in these patients were limited to autopsy material until the advent of per oral biopsy (12, 26 33) Recently using the per oral technique Stanfield *et al* and Burman Cook & Lee reported jejunal mucosal changes and disaccharidase activities in African children with kwashiorkor (6 10 31) *Brunser et al* described jejunal mucosa of malnourished infants with special reference to the mitotic index (5) Since we could find no reports on the histologic characteristics and disaccharidase levels of the intestinal mucosa in infants with marasmus, we are presenting here the results of our study of 26 such malnourished infants

MATERIAL AND METHODS

The patients were all seen at Hacettepe Children's Hospital in Ankara Turkey between September 1965 and September 1966 They all had malnutrition associated with other acute diseases such as pneumonia gastroenteritis etc The degree of malnutrition was estimated according to a modification of the criteria of Gomez *et al* for Mexican children (14) There were 26 patients (13 males and 13 females) whose ages varied from nine months to three years Four of these patients had first degree malnutrition ten had second degree seven had third degree and five fourth degree A detailed history was obtained for each one with special emphasis on his nutritional state Usual methods were used for the haematological tests (7) In some patients serum iron unsaturated iron binding capacity in the serum and serum folic acid levels were measured (17 20 29) Serum proteins were measured by the biuret method (15) D Xylose absorption tests were done on fourteen patients and oral glucose tolerance tests on thirteen by using standard described methods (18 22 34) The glucose tolerance curve is considered normal when a 30 mg increase over the fasting level in 30 min and a return to the original level within two hours In a diabetic curve the blood sugar increases to over 160 mg and does not return to the original level within two hours

Jejunal biopsies were performed by a technique previously described elsewhere using a paediatric size (8 mm × 17 mm) Crosby capsule (2) The specimen was examined under a hand loop before being pinned out on a card mucosal surface uppermost and fixed in a 10 per cent buffered formalin No examinations were done under the dissecting microscope After fixation the specimens were imbedded in paraffin wax and sections were cut and stained with haematoxylin and eosin and by the periodic acid Schiff technique Care was taken to prevent tangential cuts of the specimen None of the infants were acutely ill or suffering from gastroenteritis at the time of biopsy

In the histologic examination of the mucosa 1) height of the villi 2) epithelial cells of the villi 3) depth of the crypts and 4) cellularity and/or oedema of the lamina propria were carefully checked In normal jejunal mucosa the villi are finger like or long the epithelial cells are columnar with basilarly and regularly arranged nuclei and existing microvilli

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1 *Clinical and haematological data*

	Age (months) and sex	Weight (kg) Degree of malnutrition	Serum proteins (g/100 ml) alb/glob	Haemoglobin (g/100 ml)	Serum iron Serum iron binding Cap (γ/100 ml)
	30/♂	6 3/IV	$7\,8=\frac{5\,3}{2\,5}$	6 75	—
	36/♂	6 8/IV	$6\,3=\frac{3\,8}{2\,8}$	6 65	40/333
	30/♀	6 71/IV	$6\,5=\frac{4\,4}{2\,1}$	8 45	—
	18/♀	5 5/IV	$7\,2=\frac{4\,6}{2\,6}$	3 0	136/312 5
	24/♂	5 83/IV	—	6 95	—
	18/♀	6 38/III	$6\,4=\frac{4\,2}{2\,2}$	8 45	80/390
	18/♂	6 7/III	$4\,6=\frac{1\,5}{3\,1}$	7 40	—
	27	10 5/I	$6\,0=\frac{3\,8}{2\,2}$	10 80	—
	18/♀	6 24/III	$4\,2=\frac{2\,2}{2\,0}$	7 17	90/390
	25	10 8/—	$6\,8=\frac{4\,2}{2\,6}$	11 55	—
	36/♀	8 5/III	$6\,4=\frac{4\,4}{2\,0}$	3 85	28/314
9	42	10/—	$5\,6=\frac{3\,0}{2\,6}$	12 30	41/417
0	9/○	4 8/III	$3\,4=\frac{1\,8}{1\,6}$	9.20	—
1	10/♂	5 9/III	$6\,1=\frac{3\,9}{2\,2}$	10 05	—
12	14/♂	5 8/III	—	9 20	—
13	14/♂	7 5/II	$7\ =\frac{5\,5}{1\,5}$	9 20	67/334
	19 5	10 1/I	—	10 80	—
14	30	10 3/II	$4\,5=\frac{2\,5}{2\,0}$	6 95	64/350
15	12/♂	6 3/II	$7\,5=\frac{5\,5}{2\,0}$	9 50	—
16	30 ♀	8 7/II	$6\,5=\frac{4\,5}{2\,0}$	11 55	—
17	24/	7 5/II	$6\,8=\frac{4\,1}{2\,7}$	11 40	—
18	18/♂	8 1/II	$6\,8=\frac{3\,8}{3\,0}$	12 30	87/390
19	12	6 5/II	$6\,6=\frac{4\,2}{2\,4}$	9 6	95/346
0	18 ♂	7 8/II	$6\,8=\frac{3\,8}{3\,0}$	11 25	—
1	36	9 9 II	$4\,8=\frac{2\,8}{2\,0}$	5 45	42/408
	40	11 5/I	$6.2=\frac{4\,0}{2\,2}$	10 05	—
22	20	8 7/II	$6\,2=\frac{4\,0}{2\,2}$	8 30	183/248
3	4 3	9 3 I	$4\,2=\frac{2\,2}{2\,0}$	6 95	157/350
	27	13/—	$7\,0=\frac{4\,5}{2\,5}$	11 85	—
4	36/	12/I	$6.2=\frac{3\,7}{2\,5}$	9 65	49/350

Table 1 (cont)

Case no	Age (months) and sex	Weight (kg) Degree of malnutrition	Serum proteins (g/100 ml) alb/glob	Haemoglobin (g/100 ml)	Serum iron Serum iron binding Cap (γ/100 ml)
	44	15/—	$6.5 = \frac{3.8}{2.7}$	8.45	—
25	24/♀	10.0/I	$4.5 = \frac{2.6}{1.9}$	8.90	—
	31	12.2/—	$6.2 = \frac{3.4}{2.8}$	8.00	48/364
26	10/♀	6.33/I	—	6.80	—

Second admission — = Not done (—) = negative

the crypts show no increase in depth and the lamina propria exhibits no oedema or hypercellularity. Deviations from these characteristics are considered to be abnormal stemming from a mild to severe degree of alteration. Slight broadening or branching of the villi with areas of cuboidal cells and some degree of atrophy, increased depth of crypts, slight increase in the cellular infiltration of the lamina propria indicate mild histologic changes. Flat or short villi with atrophy in the epithelium, increase in the cellular infiltration of the lamina propria are considered as severe changes. The terms of partial or subtotal villous atrophy were not used in this study as it has been claimed that they do not properly describe histologic changes (23). Each section was assessed at least twice with consistent results. Disaccharidase activities were determined by a micro method (8) which is a modification of Dahlquist's technique (13). For this purpose the biopsy specimen was cut into two pieces: the first half was frozen in dry ice immediately and was kept at −20 C in the freezer until the assay was performed. The second half was fixed in buffered formalin for histological interpretation. At the time of assay the frozen biopsy specimen was homogenized in a sintered walled all glass homogenizer. The protein content was determined by the Warburg method (9). The estimation of invertase, lactase and maltase was carried out on a microscale by estimating the glucose liberated from these disaccharides by the hydrolases. For this purpose a purified yeast hexokinase and glucose 6 phosphate dehydrogenase coupled system was used (8). Biopsy specimens weighing as little as 0.07–0.1 mg were enough to obtain a satisfactory answer to the presence of hydrolase in the mucosa. The activities were expressed in units as micromoles of disaccharide hydrolized per milligram of protein content of biopsy specimen per hour. The assay was conducted at room temperature which varied between 25-27 C. Normal values in our laboratory for lactase, maltase and invertase are 0.112 (0.050–0.130) units/hr/mg protein, 0.295(0.250–0.340) units/hr/mg protein, 0.166(0.130–0.190) units/hr/mg protein respectively.

Seven children without malnutrition between the ages of 12 and 36 months formed a control group.

RESULTS

The age, sex, weight, degree of malnutrition and the laboratory findings are summarized in



Fig. 1. Appearance of the jejunal mucosa in an 18 month old child with third degree of malnutrition (case 7). Note that villi are broad with areas of atrophy in epithelium. The lieberkuhn crypts are elongated. Lamina propria contains increased number of mononuclear cells (lymphocytes, plasma cells and some eosinophils) and exhibits some mild oedema.



Fig 2 Repeat biopsy of the same case 9 months after therapy The villi appear finger like the epithelial cells are columnar lamina propria exhibits no oedema or hypercellularity

Table 1 Seven of the 23 patients whose serum protein levels were measured had total protein levels of 4 8 g/100 ml or less Only five patients had albumin values below 2 5 g/100 ml Their haemoglobin values varied from 3 g to 12 30 g/100 ml All but four were anaemic with haemoglobin levels under 10 50 g/100 ml After treatment these values improved rapidly In fifteen cases bone marrow showed normoblastic erythroid hyperplasia Serum iron was less than 70 μg/100 ml with a high iron binding capacity in eight of fourteen patients on whom the test was performed Serum folic acid levels were determined for eleven patients and all were found to be normal Traces of occult blood were found in the stool of five patients and two patients stool examinations revealed ascariasis The glucose tolerance was flat in one (case 24) of the twelve patients on whom it was performed another patient (case 15) had a diabetic curve The D Xylose absorption test was normal in all of the thirteen patients tested A total of 34 jejunal biopsies were taken including the eight repeat biopsies No major biopsy complications such as diffuse haemorrhage or perforation occurred In twelve patients occult blood was positive for 24–48 hours after the biopsy Seventeen specimens showed mild abnormalities in the mucosa characterized by broader and shorter villi with a tendency to branch areas of cuboidal cells and some degree of atrophy increased depth of crypts increased cellular infiltration (consisting of lymphocytes eosinophils and plasma cells) and oedema of the lamina propria (Fig 1) The mucosa in seven patients was normal Two specimens were considered insufficient for histologic evaluation

Table 2 *Absorption tests disaccharidase levels and histology*

Case no	Degree of malnutrition	Absorption tests: Glucose tol	D-Xylose	Disaccharidase levels: Lactase	Maltase	Invertase	Histology of the jejunal mucosa
	IV	N	N	0 000	0 030	0 030	Ab
3	IV	—	N	0 000	0 007	0 030	Insufficient
15	II	Diabetic	N	0 002	0 010	0 010	Ab
16	II	—	N	0 052	0 010	0 070	N
17	II	N	—	0 074	0 060	0 050	Ab
18	II	N	N	0 000	0 070	0 020	Insufficient
19	II	N	—	0 021	0 090	0 058	Ab
20	II	N	N	0 000	0 080	0 072	Ab
21	II	N	N	0 003	0 340	0 016	N
	I	—	—	—	—	—	N

Second admission Ab = Abnormal N = normal

Table 1 (cont)

Case no	Age (months) and sex	Weight (kg) Degree of malnutrition	Serum proteins (g/100 ml) alb /glob	Haemoglobin (g/100 ml)	Serum iron Serum iron binding Cap (γ/100 ml)
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25	24/♀	10 0/I	$4\,5=\frac{2\,6}{1\,9}$	8 90	—
	31	12 2/—	$6\,2=\frac{3\,4}{2\,8}$	8 00	48/364
26	10/♀	6 33/I	—	6 80	—

Second admission — = Not done (−) = negative

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Repeat biopsy specimens showed that the appearance of the mucosa returned to normal in 4 patients (cases 7 8 13 and 24) after correction of their anaemia and malnutrition (Fig 2) In these patients the intervals between the first and second biopsy were 9 7 5 5 and 8 months respectively Cases 9 21 23 who initially had normal mucosae also showed normal histology in repeat biopsies performed 6 4 and 3 5 months after the first one In one patient (case 25) the abnormal histology persisted and could be seen in the second biopsy which was done seven months later At this time, the malnutrition was corrected but the patient was still anaemic

Disaccharidase assays were performed in nine patients (Table 2) All had decreased activities of lactase maltase and invertase compared to normal controls The different enzyme activity levels varied as follows Lactase from 0 000 to 0 052 (mean 0 011 unit) maltase from 0 007 to 0 340 (mean 0 071 units) and invertase from 0 010 to 0 072 (mean 0 039 units)

The absorption tests and jejunal biopsies on seven control cases revealed no abnormalities

DISCUSSION

The results of this study indicate that the jejunal mucosa in malnourished children exhibits some mild histologic changes accompanied by a decrease in disaccharidase activities Histologic changes seen in our cases were similar to those found by Stanfield *et al* (31) and Burman (6) in African children with kwashiorkor Sprinz and his co-workers also described the same changes in Thai people from a low socioeconomic background (27) Tropical sprue (1) hookworm anaemia and iron deficiency anaemia in infants (19) and children (3 16) may cause similar histologic changes in the intestinal mucosa, the most severe is being described in coeliac sprue (4 27) Brunser and co workers (5) reported mild histologic changes in four and more marked changes in one of the 18 marasmic infants studied

In this series 6 of the ten infants with kwashiorkor exhibited a completely flat mucosa, an other 3 had flat areas with a few short villi

Stanfield *et al* (31) noted the persistence of mucosal changes in follow up studies one year after treatment for kwashiorkor In their series, there was rapid clinical improvement and complete biochemical recovery but the jejunal mucosa remained abnormal Cook & Lee (10) studied African children who had had kwashiorkor four years or more previously and follow up biopsies in 18 of these children revealed that microscopic alterations in jejunal mucosa converted to normal in most of them

In our repeat biopsies it was observed that these non specific mucosal alterations were reversible However in a 24 month old girl with first degree malnutrition (case 25) mucosa still showed the same abnormalities 7 months after her initial examination at which time she was not malnourished but had iron deficiency anaemia

There was no correlation betwen the jejunal mucosal changes and anaemia hypoproteinemia or severity of malnutrition There was also no parallellism between the results of absorption tests and intestinal histology

Multiple factors such as deficiencies of proteins iron and folic acid may possibly responsible for the above mentioned alterations in jejunal mucosa Protein is the main substance in the structure of the enzymes which are essential for the function and integrity of epithelial cells Naiman *et al* (19) described histologic changes similar to those seen in malabsorption syndromes in the duodenal mucosa of infants with iron deficiency anaemia After iron treatment, the mucosa became normal in these infants and children Recently Guha *et al* (16) demonstrated the presence of mild to severe histological abnormalities in children with iron deficiency anaemia resembling those seen in malabsorption states Patients with folic acid deficiency often develop diarrhoea and this is also reported in cases of tropical sprue with typical mucosal changes and malabsorption (1)

However protein iron and/or folic acid deficiency may not be the only factors influencing the intestinal mucosa There may also be some mucosal trophic factors or a specific trace mucosal factor present in the diet (11) Addition of these missing factors to the patient's diet will generally correct the condition of the mucosa It is claimed that an environment relatively deficient in protein could enable the mucosa gradually to return to normal (11) Mucosal changes may however be irreversible as observed in adults of low socio-economic groups in Thailand and among normal adults in Uganda and Southern India (1 4 30) After exclusion of a genetic basis for these changes it has been suggested that the persistence of mucosal change in adulthood may be the end result of a long standing chronic low protein diet in infancy or childhood (31)

In this study lactase maltase and invertase in the jejunal mucosa of nine patients were markedly decreased in comparison with normal controls The levels were at least 3 5–4 times reduced in all enzymes Here the disaccharidase deficiency might be related to malnutrition and alteration in the jejunal epithelium Stanfield *et al* (31) found low disaccharidase levels in children with kwashiorkor but with remission of the disease enzyme levels may improve remarkably (10) We had no repeat disaccharidase assays done after the initial ones in the nine infants of this series With older malnourished children our experience was similar to that of Stanfield *et al* (31) and Cook & Lee (10) Absorption tests intestinal biopsies and disaccharidase assays were carried out in four children aged 4 to 6 years Two had second degree and the other two had fourth degree malnutrition They had normal glucose tolerance curves All had decreased lactase maltase and invertase activities ranging from 0 000 to 0 030 0 096 to 0 190 0 040 to 0 090 units respectively Patients with fourth degree malnutrition showed abnormal jejunal histology which was still present 7 and 9 months after initial examination at which time they were less malnourished Disaccharidase assays showed some improvement in one Only invertase activity was found to be increasing in the other

It is reported that lactase is more severely depressed than other disaccharidases and is slow to recover in coeliac sprue and tropical sprue after treatment (21 25 28) We also found that lactase levels were the lowest in our 9 marasmic infants However the ratios of lactase to maltase and lactase to invertase were constant as expected in secondary disaccharidase deficiencies (31 32) The return of lactase activity to normal may be slower in malnourished children who exhibit a high degree of lactase intolerance Finally it should be mentioned that these secondary deficiencies may very well be the cause of chronic diarrhoeas and food intolerance in many marasmic children

SUMMARY

Twenty six infants with malnutrition and seven controls were studied by routine haematological tests serum protein levels glucose tolerance and D Xylose absorption tests jejunal biopsies and intestinal disaccharidase assays In conclusion it can be said that in malnutrition the jejunal mucosa shows some mild or non specific histologic changes compatible with malabsorption which may be reversible No correlation could be shown between these changes and absorption tests Malnutrition may also result in secondary intestinal disaccharidase deficiencies which improve with correction of the nutritional state These secondary enzyme deficiencies may be responsible for some of the diarrhoeas seen in marasmic children No direct correlation between anaemia iron-deficiency folic acid levels protein deficiency and intestinal mucosal changes could be established in this study

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We are indebted to Dr Pinar Özand and his colleagues for disaccharidase determination on biopsy specimens

REFERENCES

1 Baker S J Ignatius M Mathan V I Vaish S K & Charko C C Intestinal biopsy in tropical sprue *Ciba Symposium Study Group in Intestinal Biopsy* Basle 1962 p 84
2 Berkel I & Kiran Ö Importance of the per oral intestinal biopsy in children as a diagnostic aid (in Turkish) *Paper presented at the IVth Seminar of the Turkish Ped Soc July 1964* Istanbul
3 Berkel I Say B & Kiran Ö Intestinal mucosa in children with pica and iron deficiency anaemia (in preparation)
4 Booth C C Stewart J S Holmes R & Brackenbury W *Dissecting microscope appearance of intestinal mucosa* Intestinal Biopsy Ciba Found Study Group 14 J A Churchill Ltd London 1962 p 2
5 Brunser O Reid A Monckeberg F Maccioni A & Contreras I Jejunal biopsies in infant malnutrition with special reference to mitotic index *Pediatrics 38* 605 1966
6 Burman D The jejunal mucosa in kwashiorkor *Arch Dis Child 40* 526 1965
7 Cartwright G E *Diagnostic laboratory hematology* Grune and Stratton Inc New York 1958 2nd ed p 34
8 Ciliv G & Özand P Measurement of invertase maltase and beta galactosidase activities on small samples of intestinal mucosa *Turkish J Ped 7* 204 1965
9 Colowick S P & Kaplan N O *Methods in enzymology* Vol 3 Acad Press Inc New York 1957 vol 3 p 451
10 Cook G C & Lee F D The jejunum after kwashiorkor *Lancet II* 1263 1966
11 Creamer B Small intestinal mucosal dynamics and environment *Brit Med J II* 1373 1964
12 Crosby W H & Kugler H W Intraluminal biopsy of the small intestine the intestinal biopsy capsule *Am J Digest Dis 2* 236 1957
13 Dahlquist A Method of assay of intestinal disaccharidases *Anal Biochem 7* 18 1964
14 Dogramaci I & Wray J Severe infantile malnutrition and its management *Turkish J Ped 1* 129 1958
15 Frater H J *Manual of standardized procedures for spectrophotometric chemistry* Sci Supply Corp Pub New York P A 10(1-3) G 15(1-3) and P 50a(1-6) 1950
16 Guha D K Walia B N S Tandon B N Deo M G & Ghai O P Small bowel changes in irondeficiency anaemia of childhood *Arch Dis Child 43* 239 1968
17 Herbert V Fisher R & Koontz B J The assay and nature of folic acid activity in human serum *J Clin Invest 40* 81 1961
18 Meites S & Faulkner W S *Manual of practical and general procedures in clinical chemistry* C C Thomas Pub Springfield Ill 1962 p 231
19 Naiman J L Oski F A Diamond L K Vawter G F & Schwachman H The gastrointestinal effects of iron-deficiency anemia *Pediatrics 33* 83 1964
20 Peters T Giovanniello T J Apt, L & Ross, J F A new method for the determination of serum iron binding capacity *J Lab Clin Med 48* 274 1956
21 Plotkin G R & Isselbacher K J Secondary disaccharidase deficiency in adult celiac disease (non tropical sprue) and other malabsorption states *New Eng J Med 271* 1033 1964
22 Rapoport M Glucose tolerance test (oral) *In* Nelson (ed) *Textbook of Pediatrics* W B Saunders Co Philadelphia 1959 7th ed p 698
23 Rubin C E Dobbins W D Peroral biopsy of the small intestine A review of its diagnostic usefulness *Gastroenterology 49* 676 1965
24 Scrimshaw N S & Behar M Malnutrition in underdeveloped countries *New Eng J Med 27?* 137 1965
25 Sheehy T W Anderson P R & Baggs B E Carbonhydrate studies in tropical sprue *J Indian Med Prof 12* 5432 1965
26 Shiner M Duodenal biopsy *Lancet I* 17 1956
27 Shiner M & Doniach I Histopathologic studies in steatorrhea *Gastroenterology 38* 419 1960
28 Shmerling D H Auricchio S Rubino A Hadorn B & Prader A Der sekundäre Mangel an intestinaler Disaccharidaseaktivitat bei der Coliakie *Helvet Paed Acta 19* 507 1964
29 Simmonds J S & Gentzkow C J Determination of serum iron *Med Pub Health Met* Lea and Febiger Philadelphia 1955 p 417
30 Sprinz H Sribhibhadh R Gangarosa E J Benyajati C Kundel D & Halstead S Biopsy of small bowel of Thai people with special reference to recovery from Asiatic cholera and to an intestinal malabsorption syndrome *Amer J Clin Path 38* 43 1962
31 Stanfield J P Hutt M S & Tunnicliffe R Intestinal biopsy in kwashiorkor *Lancet II* 519 1965
32 Townley R W Disaccharidase deficiency in infancy and childhood *Pediatrics 38* 127 1966
33 Trowell H C Davies J N P & Dean R F A *Kwashiorkor* Edward Arnold and Co London 1954 p 149
34 Varley H Blood sugar and its determination method of Folin and Wu *Pract Clin Biochem* William Heineman Med Books Ltd London 1958 2nd ed p 33

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CHROMOSOMAL CONDITIONS IN CONGENITAL HEART DISEASE

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Congenital cardiovascular disease is often found as an associated malformation in patients with various chromosomal abnormalities It is thus a well known observation that in patients with Down's syndrome Turner's syndrome cri du chat trisomy D and trisomy E congenital heart disease shows a far greater incidence than in normal subjects However congenital heart disease also occurs frequently together with other malformations in patients showing more unusual chromosomal abnormalities such as partial trisomy in groups A B and C

Chromosomal abnormalities in a mother and son who both had a congenital heart disease —atrial septal defect—as sole malformation (2) were reported for the first time in 1961 Since then several chromosomal studies have been published in a large number of patients with congenital heart disease with or without other accompanying malformations Thus Sasaki et al (13) examined the chromosome picture in 22 patients with congenital heart disease Three patients presented a clinical picture of Down's syndrome with trisomy G Eleven patients were cytogenetically normal In 9 patients (one of whom also had trisomy G) chromosome no 16 showed differences in size presenting a picture of monosomy for no 16 and trisomy for no 19 Such a heterozygous condition for pair no 16 varied in degree even in the same patient

Anders *et al* (1) examined 156 patients with congenital heart disease patients with Down's syndrome and Turner's syndrome being excluded from the material Among these 156 patients none were found with major chromosomal abnormalities *Five patients* however showed minor variations in the size of individual chromosomes For example in 2 patients one chromosome no 16 was as large as no 12 The increased length was apparently due to the elongation of the *secondary* constriction normally found in the paracentric region of the long arms *In another* 2 patients the majority of the cells showed one 16 shorter than usual and in extreme instances it resembled a 19 In the fifth patient the short arms on a *chromosome 15* were unusually long Family members were examined in 2 of *the cases* and some of them showed the same chromosomal abnormalities as the propositus although not resulting in congenital heart disease

In 1966 Rohde (11) published 68 cases with heart disease where the karyotype had been studied The author had been particularly interested in patients with accompanying congenital malformations ovarian and testicular dysgenesis and certain mental abiotrophic and neurological disorders Eleven patients were representative of the clinical syndromes associated with simple or partial autosomal trisomia in groups C D E and G and 10 patients ha

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REFERENCES

1 Baker S J Ignatus M Mathan V I Vaish S K & Charko C C Intestinal biopsy in tropical sprue *Ciba Symposium Study Group in Intestinal Biopsy* Basle 1962 p 84
2 Berkel I & Kiran Ö Importance of the per oral intestinal biopsy in children as a diagnostic aid (in Turkish) *Paper presented at the IVth Seminar of the Turkish Ped Soc July 1964* Istanbul
3 Berkel I Say B & Kiran Ö Intestinal mucosa in children with pica and iron deficiency anaemia (in preparation)
4 Booth C C Stewart J S Holmes R & Brackenbury W *Dissecting microscope appearance of intestinal mucosa* Intestinal Biopsy Ciba Found Study Group 14 J A Churchill Ltd London 1962 p 2
5 Brunser O Reid A Monckeberg F Maccioni A & Contreras I Jejunal biopsies in infant malnutrition with special reference to mitotic index *Pediatrics 38* 605 1966
6 Burman D The jejunal mucosa in kwashiorkor *Arch Dis Child 40* 526 1965
7 Cartwright G E *Diagnostic laboratory hematology* Grune and Stratton Inc New York 1958 2nd ed p 34
8 Cilıv G & Özand P Measurement of invertase maltase and beta galactosidase activities on small samples of intestinal mucosa *Turkish J Ped 7* 204 1965
9 Colowick S P & Kaplan N O *Methods in enzymology* Vol 3 Acad Press Inc New York 1957 vol 3 p 451
10 Cook G C & Lee F D The jejunum after kwashiorkor *Lancet II* 1263 1966
11 Creamer B Small intestinal mucosal dynamics and environment *Brit Med J II* 1373 1964
12 Crosby W H & Kugler H W Intraluminal biopsy of the small intestine the intestinal biopsy capsule *Am J Digest Dis 2* 236 1957
13 Dahlquist A Method of assay of intestinal disaccharidases *Anal Biochem 7* 18 1964
14 Dogramacı I & Wray J Severe infantile malnutrition and its management *Turkish J Ped 1* 129 1958
15 Fister H J *Manual of standardized procedures for spectrophotometric chemistry* Sci Supply Corp Pub New York P A 10(1-3) G 15(1-3) and P 50a(1-6) 1950
16 Guha D K Walia B N S Tandon B N Deo M G & Ghai O P Small bowel changes in irondeficiency anaemia of childhood *Arch Dis Child 43* 239 1968
17 Herbert V Fisher R & Koontz B J The assay and nature of folic acid activity in human serum *J Clin Invest 40* 81 1961
18 Meites S & Faulkner W S *Manual of practical and general procedures in clinical chemistry* C C Thomas Pub Springfield Ill 1962 p 231
19 Naiman J L Oski F A Diamond L K Vawter G F & Schwachman H The gastrointestinal effects of iron deficiency anemia *Pediatrics 33* 83 1964
20 Peters T Giovanniello T J Apt L & Ross J F A new method for the determination of serum iron binding capacity *J Lab Clin Med 48* 274 1956
21 Plotkin G R & Isselbacher K J Secondary disaccharidase deficiency in adult celiac disease (non tropical sprue) and other malabsorption states *New Eng J Med 271* 1033 1964
22 Rapoport M Glucose tolerance test (oral) In Nelson (ed) *Textbook of Pediatrics* W B Saunders Co Philadelphia 1959 7th ed p 698
23 Rubin C E Dobbins W D Peroral biopsy of the small intestine A review of its diagnostic usefulness *Gastroenterology 49* 676 1965
24 Scrimshaw N S & Behar M Malnutrition in underdeveloped countries *New Eng J Med 272* 137 1965
25 Sheehy T W Anderson P R & Baggs B E Carbonhydrate studies in tropical sprue *J Indian Med Prof 12* 5432 1965
26 Shiner M Duodenal biopsy *Lancet I* 17 1956
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28 Shmerling D H Auricchio S Rubino A Hadorn B & Prader A Der sekundäre Mangel an intestinaler Disaccharidaseaktivität bei der Coliakie *Helvet Paed Acta 19* 507 1964
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Table 1 *Age sex chromatin and distribution of chromosome counts for 82 patients with congenital cardiovascular disease*

Ref no	Age (yrs)	Sex chromatin (positive)	Chromosome counts <45	45	46	47	>47	Total cells
12	4	3		1	28	1		30
13	1 5/12	2		2	48			50
19	3	27	1	5	38		1	45
4	5/12	40	2	3	34	1		40
37	11/12	5	3	8	25			36
41	2	45	3	2	28	1		34
42	1	9	4	1	38			43
44	1	9	1	2	38			41
45	5	5	1	2	31			34
49	6/12	3		3	36	1		40
54	4/12	2	5	3	33			41
55	1/12	31	1	3	37			41
57	1/12	6	2	5	32			39
59	6	34	1	5	35			41
61	1	3	3	6	36			45
66	0/12	2	1	1	34			36
67	1/12	1			38	1		39
68	8/12	1	7	5	40			57
74	3/12	32	13	4	41			58
75	4/12	5	1	5	34			40
81	1/12	5	4	4	42	1		51
87	6/12	8	3	2	30			35
89	4	36		3	37			40
91	0/12	31	1	4	38			43
92	2	33		3	37			40
93	1	1	2	7	37			46
94	10 12	32	2	5	51	2		60
103	[illegible]/12	33	1	2	28			31
104	1	30	2	3	36			41
105	3/12	1	5	11	34			50
108	3	40	6	3	41			50
109	2	27	3	2	35			40
110	0 12	4	2	2	46			50
112	10/12	25	1	2	38			41
117	2/12	28		1	40			41
119	9/12	31			50			50
125	0 12	30	2	6	32			40
126	2	31	1	3	42			46
127	1/12	1	3	3	44			50
128	10 12	36	1	5	44			50
129	10	2		1	45			46
134	4	44	2	4	44			50
137	3/12	38	4	6	36			46
138	5	2	1	2	47			50
139	3/12	4	4	5	37			46
142	3 12		2		48			50
143	2	24		3	37			40
144	5 12	4	1	12	60			73
145	1	5		2	38	1		41
146	3 12	5		1	49			50
147	5/12	74		3	40			43
149	1	3	3	1	41			43
155	11	4	3	5	33			41
158	10	40	2	4	32	1		39
159	10	—	2	2	37			41
161	7	31	1	4	37			42
163	6	1	2	4	36			42
164	1	1	1	3	36			40
166	10	19	4	3	50			57
171	6	38		1	49			50
177	5 1	3	1	5	47			48
183		2	1		39			40

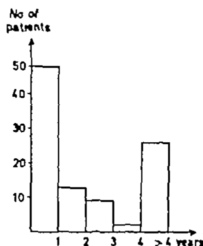


Fig 1 The age distribution

abnormalities of the sex chromosomes In 34 patients with congenital heart disease and in 13 patients with cardiovascular disorders, normal chromosomal conditions were found

Emerit *et al* (6) examined 275 patients with congenital heart disease, selected either for the presence of extracardiac abnormalities or the existence of congenital heart disease in other members of the family Thirty nine patients had classical chromosomal abnormalities Sixteen other patients had minor autosomal abnormalities In numerous cases these abnormalities were found in only a few of the cells examined The karyotypes of a few of the family members were also examined In one patient, for example 10 out of 14 cells showed a small acentric chromosome fragment which was not found in the mother In another patient and his mother a constant break was found in the long arm of a group C chromosome In 2 brothers both with congenital heart disease, a chromosome in the C group was found to be abnormally large and in many of the cells this chromosome showed a secondary constriction The same abnormality was also found in the mother and in another brother although they did not show any congenital malformations

MATERIAL AND METHODS

Many children are admitted annually to Queen Louises Childrens Hospital for examination and treatment for congenital cardiovascular disease It was therefore possible to carry out an extensive investigation as to whether any relationship could be demonstrated between chromosomal abnormalities and congenital cardiovascular disease—and in particular whether there were variations in the size of the chromosomes also in this material described in some of the studies mentioned above

The investigation took place during the years 1964–67 the material consisting of 100 patients in whom the diagnosis of cardiovascular disease was confirmed by cardiac catheterization angiocardiography or operation In some cases of persistent ductus arteriosus where the diagnosis was so obvious that cardiac catheterization was unnecessary the diagnosis was verified by operation alone The material includes practically all forms of congenital heart defect and patients who also had other malformations or known syndromes were not excluded from the material

The karyotype was studied on blood cultures prepared according to Frøland (7) a micromethod based on the principles of Moorhead *et al* (1960) Nuclear sex was determined on buccal smears stained by Feulgen method In our laboratory about 200 sex chromatin determinations were made in patients without sex chromosome abnormalities In this material less than 9 of the cells in the buccal smear in the case of phenotypic boys were found to contain a chromatin body (= sex chromatin negative) while in the case of phenotypic girls more than 19 of the cells were found to contain a chromatin body (= sex chromatin positive)

RESULTS

Fifty four out of the 100 patients were phenotypic girls Of these 50 were sex chromatin positive, while 4 were sex chromatin negative These 4 showed clinical signs of Turners syndrome with a karyotype 45/X0 The remaining 46 patients were phenotypic boys 45 of whom were sex chromatin negative (In 1 patient (no 142) the sex chromatin determination failed and the child died before a new investigation could be made)

Fig 1 shows the age distribution Half of the patients were less than 12 months old at the time of investigation and 39 were even less than 6 months old

In 82 out of the 100 patients no major chromosome abnormalities were found The chromosome investigations showed a modal number of 46 and the analysis revealed a normal karyotype for the sex Age nuclear sex

Table 3. *Age, sex chromatin and distribution of chromosome counts in 4 patients with congenital cardiovascular disease and Turner's syndrome*

Ref. no.	Age (yrs)	Sex chromatin (% positive)	Chromosome counts <45	45	46	47	>47	Total cells
16	1	4	7	50				57
249	17	1	6	35				41
270	12	0	2	49				51
304	2	4	1	39				40

Table 4. *Age, sex chromatin and distribution of chromosome counts in 2 patients with congenital heart disease and chromosome abnormalities*

Ref. no.	Age (yrs)	Sex chromatin (% positive)	Chromosome counts <45	45	46	47	>47	Total cells
123	3/12	6	2	2	45			49
189	1/12	1	5	5	46	1		57

negative with a karyotype 45/X0 without mosaics m (Table 3).

Two patients, on the other hand, had unusual karyotypes (Table 4), for which reason they will be described in more detail. The first patient (no. 123) was a 3 month old boy. An elder brother as well as both parents were healthy and phenotypically normal. The parents were unrelated, and there were no malformations in the family. The father was 27 years old and the mother 25 years old when the patient was born. The mother had never aborted. Pregnancy and delivery were normal. Birth weight was 3500 g and birth length 54 cm. After birth contractures of the fingers were observed as well as a single crease on either little finger. From birth there was occasionally dyspnoea and later attacks of cyanosis and apnoe. A roentgenogram of the thorax, cardiac catheterization and operation showed retro-oesophageal vessels, patent ductus arteriosus, anomalies of pulmonary venous return and right aortic arch. Autopsy confirmed the above diagnoses but showed no further malforma-

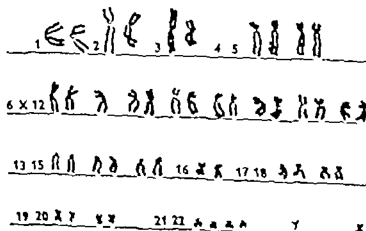


Fig. 2. Karyotype of patient no. 123 with a Y isochromosome or pericentric inversion of the Y chromosome.

Table 1 (*cont*)

Ref no	Age (yrs)	Sex chromatin (positive)	Chromosome counts <45	45	45	47	>47	Total cells
187	10/12	39	4	4	38	1		47
194	1/12	40	3	2	40			45
195	20	40	1	5	50	1		57
196	21	33		1	43			44
200	43	28		2	38			40
201	23	19	2	3	36			41
205	41	2	1	3	44			48
206	1/12	2	1	2	43			46
207	2	24	1		39			40
210	35	38		3	33			36
212	25	29	1	1	33			35
215	1/12	2	2	2	47			51
219	1	27	1	1	38			40
224	30	24		1	39			40
233	9	38	1	3	37			41
263	1	18		4	47			51
276	0/12	25		4	35			39
283	0/12	36		5	35	1		41
287	6/12	1	1	2	51			54
288	5	21		2	33			35

and distribution of chromosome counts are seen in Table 1. However, two out of the 82 patients showed minor chromosomal variations. In one of these two patients (no 263) a discrepancy in the size of the two chromosomes no 16 was found in all cells. No chromosome no 16 however was as big as a chromosome in group C or as small as a chromosome in group F. No chromosome studies were made among the members of the family of this patient. In the other patient (no 94) all cells examined showed an unusually long chromosome in group C. The chromosome in questio had a secondary constriction in the pericentri region of the long arm. The karyotype of th family was not examined.

The hypomodal cells showed no constan chromosomal pattern.

Definite chromosomal abnormalities wer found in 18 out of the 100 patients. Twelv of these were phenotypic mongols, all with tri somy G without mosaicism (Table 2). Four o the 18 patients showed a clinical picture o Turner's syndrome and were all sex chromatin

Table 2. *Age, sex chromatin and distribution of chromosome counts for 12 patients with congenita heart disease and Down's syndrome*

Ref no	Age (yrs)	Sex chromatin (positive)	Chromosome counts <45	45	46	47	>47	Total cells
38	2	38	2	1	6	28		37
97	2/12	6			7	39		46
122	0/12	13	2	3	3	48		56
165	0/12	4	1	1	8	42		52
192	5/12	2		3	3	38		44
208	0/12	32	1	1	1	41		44
236	5/12	22	3	6	13	61		83
258	1/12	1	1		4	39		44
268	1/12	2	1		3	42		46
278	6/12	1			1	35		36
285	1/12	2	1		4	41	4	50
300	1	0			3	37		40

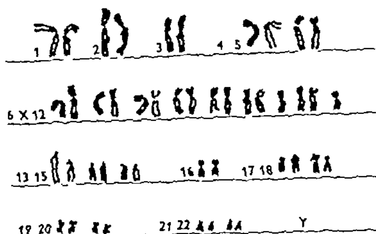


Fig 5 Karyotype of the sister of patient no 189 Translocation C/D

a broad flat nose low set ears *deformities* of the hands and feet and bilateral dislocation of the hip Cardiac catheterization showed ventricular septal defect with considerable increase in pressure in the right ventricle The patient was sex chromatin negative A chromosome investigation showed a modal number of 46 In all the cells investigated one chromosome in group D was *found to be considerably larger* than the other chromosomes in the group (Fig 4) The remainder of the chromosomes were normal The mother's karyotype was normal but both father and sister had chromosome abnormalities as like the patient they had a large chromosome in group D but in addition an unusually small chromosome presumably belonging to group C (Fig 5) It may be assumed that in the father and sister this was a case of translocation C/D with *maintenance* of the normal amount of genetic material In the propositus it may be considered that a translocation C/D is also involved but furthermore with partial trisomy for a chromosome in group C

Table 5 shows the cardiovascular malformations found in relation to the karyotypes

DISCUSSION

Chromosome preparations are subject to many mutilating factors which in individual cells

Table 5 *Cardiovascular malformations in relation to the karyotype*

Cardiovascular malformation	No of patients	Normal karyotype	Abnormal karyotype
ASD	15	13	2 trisomy G
PDA	14	14	
T of F	12	11	1 trisomy G
A V c m	3	1	2 trisomy G
C of A	5	1	4 45/XO
VSD	30	4	5 trisomy G; 1 translocation C/D partial trisomy C
Various	16	13	2 trisomy G; 1 no-Y
Type not verified	5	5	
Total	100	82	18

ASD = Atrial septal defect secundum type PDA = patent ductus arteriosus T of F = tetralogy of Fallot A-V com = persistent canal atrioventriculare commune C of A = coarctation of the aorta VSD = ventricular septal defect

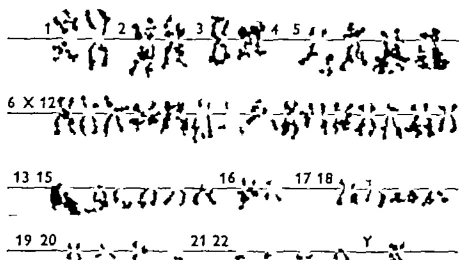


Fig 3 Autoradiography on preparations from the father to patient no 123

tions in the internal organs A determination of sex chromatin in the cells of buccal smear showed that 6 % of the cells contained a chromatin body The chromosome analyses showed a modal number of 46 (Fig 2) and all cells contained 5 chromosomes in group F and only 4 chromosomes in group G + Y The father paternal grandfather and brother of the propositus all had the same karyotype but the mother the paternal grandmother and the 2 sisters of the father had normal karyotypes Autoradiography was performed (Fig 3) on preparations from the father of the propositus and this showed that one of the chromosomes of the F group was considerably more late labelling than the others This finding together with the fact that the same chromosome abnormality was found in all male members of the family can best be explained by a Y chromosome abnormality This could either be a Y isochromosome or a pericentric inversion of the Y chromosome

The other patient with an unusual karyotype was a 2 month old boy (no 189) who was admitted for cardiac examination His chromosome anomaly has already been described by Jensen & Melchior (8) who found the following father mother and an elder sister were phenotypically normal The mother had had 2 abortions There was no known congenital malformation in the family The patient showed numerous malformations cleft palate

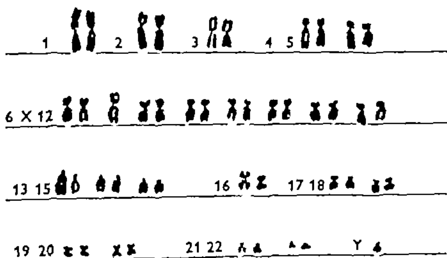


Fig 4 Karyotype of patient no 189 with translocation C/D and partial trisomy for a chromosome in group C

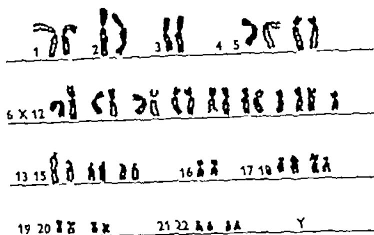


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VSD	30	24	5 trisomy G; 1 translocation C/D partial trisomy C
Various	16	13	2 trisomy G; 1 no-Y
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ASD = Atrial septal defect secundum type PDA = patent ductus arteriosus T of F = tetralogy of Fallot A-V com = persistent ostium atrioventricular commune C of A = coarctation of the aorta VSD = ventricular septal defect

may influence the size of the chromosomes as well as their number or shape The aim of the present study has not been a statistical evaluation of the incidence of chromosome breaks, heteromorphism structural rearrangements variations of satellite or aneuploidy as the occurrence of these abnormalities in individual cells may be caused by technical artefacts or may be the result of irradiation following angiocardiography or other roentgenological investigation The present material therefore only includes those chromosomal abnormalities which occur in all cells with the modal number

In the present material of 100 patient with congenital cardiovascular disease 16 patients had a classic syndrome—Down s syndrome or Turner s syndrome in which it is well known that congenital heart disease is a frequent occurrence In Down s syndrome it is most often a case of persistent ostium atrioventriculare commune atrial septal defect or ventricular septal defect In the present material 9 of the 12 patients with Down s syndrome had one of these defects The defect most often seen in Turner s syndrome is coarctation of the aorta which was present in all 4 patients with Turner s syndrome in this material

The patient (no 189) with translocation C/D and partial trisomy for a group C chromosome had in addition to heart disease numerous other congenital malformations Only a few cases have been published of presumed partial trisomy of a chromosome in group C (4 9 10 12) and a single case (5) with presumed pure trisomy of a chromosome in group C In the cases with partial trisomy balanced reciprocal translocation involving a C group chromosome occurred in one or several family members who were phenotypically normal The patients described in the above reports showed numerous different malformations and in some of them there was congenital heart disease in addition

Secondary constriction of a chromosome is often seen particularly in group C and is a phenomenon without any special significance The secondary constriction seen in all the cells in patient no 94 cannot therefore be regarded as an aetiological factor in her heart disease (persistent ductu arterio us)

It is improbable that there is any chromosomal explanation for her heart disease (ventricular septal defect) in the one patient (no 263) with a discrepancy in the size of the 2 no 16 chromosomes, as this phenomenon has often been described as an insignificant familial occurrence in otherwise normal persons (3)

In the patient with the Y chromosome abnormality other slight malformations were found in addition to the congenital heart disease—contractures of the fingers and a single crease on either little finger However as the same karyotype was found in both father brother and paternal grandfather without any accompanying malformations, this tells strongly against any relationship between the abnormal karyotype and the patient s heart disease Presumed isochromosomy for the Y chromosome was described in 1966 (14) in father and son who were not reported to have shown other malformations However both these patients were unusually tall—182 and 185 cm, respectively Another case of presumed isochromosomy was described in 1966 (3) in a man who was 152 cm tall The fact that the father and the paternal grandfather of the patient were of normal height perhaps tells in favour of the presence in this case of a pericentric inversion of a relatively large Y chromosome

With the present cytogenetic technique as well as the inability to demonstrate minimal chromosomal abnormalities microscopically the possibility cannot be excluded of a causal connection between chromosomal abnormalities and congenital cardiovascular disease In the present material however it has not been possible to reveal any chromosomal abnormality in patients with congenital heart disease where the latter was not associated with known syndromes or severe extra cardiac malformations

SUMMARY

Chromosome studies were made in 100 patients with congenital cardiovascular disease. Twelve patients had Down's syndrome with trisomy G. Four patients had Turner's syndrome with karyotype 45/XO. In one patient monochromosomy or pericentric inversion of the Y chromosome was found and was also found in the paternal grandfather, the father and the brother, none of whom had malformations. One patient who also had severe extracardiac malformations had partial trisomy of a group C chromosome and translocation C/D. Translocation C/D was found in the father and sister who were phenotypically normal. In the remaining 82 patients normal chromosomal conditions were found, although 2 patients had presumably insignificant chromosomal variations.

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REFERENCES

1 Anders, Janet M, Moores, Elizabeth C & Emanuel, R. Chromosome studies in 156 patients with congenital heart disease. *Brit Heart J 27* 756 1965

Bound, J A, Sandstrom, B & Zetterqvist, P. Association between congenital heart malformation and chromosomal variations. *Acta Paediat Scand 50* 1 1961

3 Court Brown, W M, Buckton, Karin E, Jacobs, Patricia A, Tough, Ishbel M, Kuenssberg, E V & Knox, J D D. *Chromosome studies on adults*. Cambridge University Press, Cambridge 1966

4 Edwards, J H, Fraccaro, M, Davies, Pamela & Young, R B. Structural heterozygosis in man: analysis of two families. *Ann Hum Genet 26* 163 1962

5 El Alfi, O S, Powell, H C & Biesele, J J. Possible trisomy in chromosome group 6–12 in a mentally retarded patient. *Lancet I* 700 1963

6 Emerit, Ingrid, de Grouchy, J, Vernant, P & Corone, P. Chromosomal abnormalities and congenital heart disease. *Circulation 36* 886 1967

7 Frøland, A. A micromethod for chromosome analysis on peripheral blood cultures. *Lancet II* 1281 1962

8 Jensen, M K & Melchior, J C. Possible partial trisomy of a chromosome of group 6 X 12 and familial translocation heterozygosity in a child with congenital abnormalities. *Develop Med Child Neurol 9* 313 1967

9 Lindsten, J, Fraccaro, M, Klinger, H P & Zetterqvist, P. Meiotic and mitotic studies of a familial reciprocal translocation between two autosomes of group 6-12. *Cytogenetics 4* 45 1965

10 Mikkelsen, Margareta, Mortensen, Else, Skakkebæk, N E & Yssing, Mauna. Familial reciprocal translocation between a group C (12?) chromosome and a late labelling G chromosome. *Acta Genet 18* 241 1968

11 Rohde, R A. The chromosomes in heart disease. *Circulation 34* 484 1966

12 Rohde, R A & Catz, B. Maternal transmission of a new group C (6/9) chromosomal syndrome. *Lancet II* 838 1964

13 Sasaki, M, Makino, S & Kajii, T. Chromosomal aberrations in congenital cardiovascular disorders in man. *Proc Japan Acad 39* 188 1963

14 Stoeckenius, M. A probable Y isochromosome in father and son of tall stature. *Lancet II* 391 1966

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GLUCOSE GALACTOSE MALABSORPTION

Studies on the Intermediate Carbohydrate Metabolism

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The clinical picture of glucose galactose malabsorption (GGM) has been described recently in detail (15) The condition manifests itself mainly by severe watery diarrhoea from birth This is caused by a defective absorption of the monosaccharides glucose and galactose Small amounts of glucose are however absorbed or may be transformed from other compounds in the intermediate metabolism It was therefore considered of interest to investigate whether glucose when it does enter the blood stream is normally metabolized Galactose metabolism was studied similarly

The fructose absorption is normal in GGM as observed in intestinal intubation studies (13) Remarkably oral fructose loadings usually resulted in marked increases of the blood glucose level (15) in contrast to what is normally seen It was therefore considered worth while to study this phenomenon more thoroughly

A preliminary report of these studies has been given (12)

MATERIAL

The material comprised of 3 patients and 2 normal children Some clinical data including information on the diets are given in Tables 1 and 2 The diets listed here had been fed for at least 4 days (considerably longer in most cases) prior to the different studies

Two patients cases 1 and 2 suffered from GGM Their histories have been described previously (15) then designated as cases 4 and 6 respectively

The third patient case 3 suffered from cystic fibrosis of the pancreas The diagnosis was based on a typical clinical picture including failure to thrive steatorrhea hypoproteinemia a chymotrypsin activity in the stools (9) corresponding to 10–14 μg per g faeces a sodium content of the sweat of 99 116 mEq/l and a normal xylose absorption test

Controls for the intravenous galactose tolerance tests (GalTT) were provided by two children in a good nutritional condition and without signs of digestive or metabolic diseases

METHODS

Intravenous glucose and galactose tolerance tests were performed as rapid infusions of 15 g sugar per sq m of body surface area at the second GalTT in case 2 the amount of galactose infused was however 8 4 g per sq m (0 35 g per kg body weight) The infusions were given within 2–3 min into a cubital or an external jugular vein following an overnight fast Due to lack of time the first GalTT in case 2 was however done 3 hours after a GTT The half life ($t_{½}$) of injected carbohydrates was read from a semi logarithmic plot drawn to fit as well as possible the blood concentration values obtained 15 min and onwards after the time of injection

Oral fructose tolerance tests (FTT) were performed after an overnight fast with doses of 2 g per kg body weight given as a 10 solution in water

Capillary blood samples were taken at suitable intervals for the determination of blood sugar levels Duplicate samples were taken for the determination of the fasting blood glucose level Whole blood was deproteinized using $Ba(OH)_2$ and $ZnSO_4$ (20) after

Abbreviations CHO carbohydrate FTT oral fructose tolerance test GGM glucose galactose malabsorption GalTT intravenous galactose tolerance test GTT intravenous glucose tolerance test i v intravenous $t_{½}$ half life of injected carbohydrate

Table 1. *Clinical data on patients and controls subjected to intravenous glucose tolerance and galactose tolerance tests and the resulting half life values of injected carbohydrate*

Subject	Sex	Age (months)	Height (cm)	Weight (kg)	Diet data	Calories: Daily intake	Calories: from well absorbed CHO	I.v. test $t_{\frac{1}{2}}$ in min: GTT	I.v. test $t_{\frac{1}{2}}$ in min: GalTT
Case 1 (GGM)	♀	19	80	10.2	8 g fructose per day	900	4	85	—
Case 2 (GGM)	♂	10	74	8.4	42 g fructose per day	900	20	17	—
		13	81	10.0	30 g fructose per day	1000	12	32	11
		70	120	19.9	10 g* fructose per day	1500	3	—	8
Control 1	♂	14	79	11.6	Ordinary mixed diet	1100	50	—	9.5
Control 2	♂	19	84	11.0	Ordinary mixed diet	1100	50	—	6.5

In addition the day prior to this i.v. GTT the patient received a long lasting glucose infusion for measurement of the renal reabsorption of glucose producing blood sugar levels up to 900 mg/100 ml the total amount of glucose infused was 32 g.

Ingested mainly in the shape of sucrose

centrifugation the supernatant was subjected to analysis.

Glucose was assayed using a glucose oxidase method (4). Galactose was determined in a similar way using galactose oxidase as described previously (11, 15, 1). Fructose was determined by a method specific for keto-sugars (10).

RESULTS

Fig. 1 illustrates the GTT's performed in the two patients with GGM. Case 1, who prior to the test had been living on a CHO poor diet including only 8 g per day of fructose given as 200 g of a formula containing 4 fructose, showed a slow removal rate of injected glucose (Curve I, $t_{\frac{1}{2}} = 85$ min). After 1 hour the removal rate increased but it was still slow compared with the normal values (19–45 min) for her age (14). Case 2, who when his second i.v. GTT was done was of about the same size as case 1, metabolized the injected sugar at a faster rate (Curve II, $t_{\frac{1}{2}} = 32$ min). His diet contained 30 g of fructose per day. Some months earlier he had had another GTT; at that time he consumed more fructose, 42 g

Table 2. *Clinical data on patients subjected to oral fructose tolerance tests and resulting increments of the blood glucose concentration*

Patient	Sex	Age (months)	Height (cm)	Weight (kg)	Diet and drugs	Calories: Daily intake	Calories: from CHO	1st hour blood glucose increments mg/100 ml
Case (GGM)	♂	2	59	3.8	Soy and casein formula supplying 16 g fructose per day	400	16	32
		5	68	5.5	Casein formula supplying 80 g fructose per day	800	40	9
		10	74	8.4	Mixed diet, 4 g fructose per day	850	11	33
Case 3 (cystic fibrosis)	♂	4	58	4.0	Nutramigen, no enzymes	335	50	50
		5	60	4.5	Pelargon, pancreatic enzymes	6.5	50	7

In case the values refer to well absorbed CHO (fructose). In case 3 the percentage of calories has been derived from the total CHO content of the diet.

Nutramigen (Mead Johnson) contains 8.5 carbohydrates, partly sucrose (and partly starch).

Pelargon (Nestlé), when prepared as a 17 solution in water, contains 3.7 lactose, 2.0 sucrose, 2.0 corn sugar and 1.4 starch, altogether 9.1 carbohydrates.

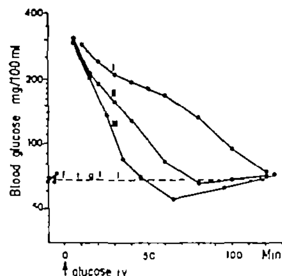


Fig 1 Intravenous glucose tolerance tests in two patients with glucose galactose malabsorption Curve I ($t_{\frac{1}{2}}$ - 85 min) case 1 19 months 4 of caloric intake consisted of fructose Curve II ($t_{\frac{1}{2}}$ = 32 min) case 2 13 months 12 of caloric intake consisted of fructose Curve III ($t_{\frac{1}{2}}$ - 17 min) case 2 10 months 20 of caloric intake consisted of fructose furthermore the day prior to the test a large intravenous infusion of glucose was given *Note* An inverse correlation between $t_{\frac{1}{2}}$ values and dietary intake of fructose was found

per day and furthermore on the day before the test he received a low infusion of 32 g of glucose[1] At that test he was found to have a fast removal rate of injected glucose (Curve III $t_{\frac{1}{2}}$ - 17 min) just within the normal range of variation for his age (14)

The results of the i v GalTT s of case 2 and of the two controls are presented in Fig 2 In case 2 at 13 months of age the elimination of galactose from the blood ($t_{\frac{1}{2}}$ - 11 min) proceeded more rapidly than the elimination of glucose but it was somewhat slower than in the controls who both had values within the normal range ($t_{\frac{1}{2}}$ < 10 min) (19) All the subjects studied however presented a moderate rise in the blood glucose concentration as a response to the galactose injection indicating that in the patient as well as in the controls some galactose was converted to glucose At 6 years of age case 2 had a normal galactose removal rate $t_{\frac{1}{2}}$ was 8 min and thus well within the normal standards of less than 12 min for children between 5 and 10 years of age (19)

The oral FTT s carried out in case 2 (GGM) and case 3 (cystic fibrosis) caused similar rises in the blood fructose concentration irrespective of the diets or treatments given (Figs 3 and 4 Table 2) The response of the blood *glucose* concentration was however variable in both cases Flat curves were obtained when the patients were given sufficient calories and an ample supply of absorbable carbohydrates in the pre test diet Higher responses were seen when the patients were deprivated of carbohydrates In case 3 at the first study (age 4 months) part of the dietary carbohydrates were badly utilized as starch could hardly be digested due to lack of pancreatic enzymes The total supply of calories was also low At this

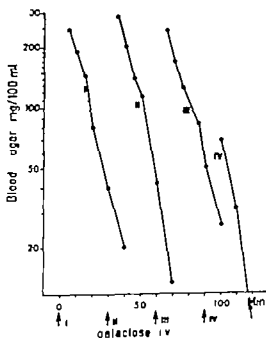
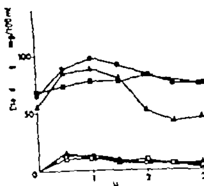


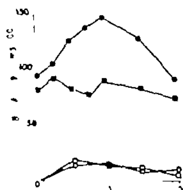
Fig 2 Intravenous galactose tolerance tests in two controls and one patient with glucose galactose malabsorption Solid lines blood galactose Interrupted lines blood glucose Curve I ($t_{\frac{1}{2}}$ - 9 5 min) Control 1 14 months Curve II ($t_{\frac{1}{2}}$ - 6 5 min) Control 2 19 months Curve III ($t_{\frac{1}{2}}$ - 11 min) Glucose galactose malabsorption case 2 10 months Curve IV ($t_{\frac{1}{2}}$ - 8 min) Same patient 6 years At the latter test a smaller dose of galactose (in relation to body size) was injected than at the other tests

[1] This infusion which augmented the blood sugar concentration up to levels of 900 mg/100 ml was given for measurement of the renal tubular reabsorption of glucose



F g 3 Oral fructose tolerance tests in a patient with glucose galactose malabsorption (case 2) Symbols 2 months 16% of dietary carbohydrate consisted of fructose △▲ 10 months 11 of dietary carbohydrates consisted of fructos □■ 5 months 40 of dietary carbohydrates consisted of fructose O en symbols blood fructose Solid symbols blood glucose Note The supranormal rise of blood glucose was abolished by increased amounts of dietary fructose and it reappeared later on when the diet contained less fructose

occasion the increase of the blood ugar concentration during the first hour was 50 mg/100 ml (Fig 4) This finding was similar to the blood sugar ri es observed in case 2 (Fig 3) when his diet contained only small amoun s



F g 4 Oral fructose tolerance tests in a patient with cystic fibrosis of the pancreas (ca e 3) Symbols 4 months, low-caloric diet and no enzyme substitution ■ 5 months sufficient caloric intake and enzymes substituted Open symbols blood fructose sol d symbols blood glucose Note The supranormal rise of blood glucose was aboli hed by improved nutrition

of fructose (1 hour increments of 32 and 33 mg/100 ml respectively above the fasting level)

DISCUSSION

The elimination curve of glu ose obtained in case 1 at 19 months of age is very similar to that een in healthy babies in the neonatal period In newborn babies the glucose tolerance is low and furthermore in the second hour of an i v GTT there is usually an increase of the glucose removal rate just as observed in case 1 (2) The low glucose tolerance of newborn babies may be a consequence of the low blood g ucose concentration of the foetus (5) Intravenous GTT's in severely malnourished infants al o demonstrate a decreased rate of glucose removal (17) In adolescent boys (18) and in adults (3) too a dietary deprivation of carbohydrates has been shown to diminish the rate of removal of administered glucose from the blood

Soon after the newborn period the glucose tolerance is higher than in adults (7 14) but later on in infancy and childhood a gradual decrea e in the glucose removal rate occurs (14) This latter development seems to be well correlated with the decrease in caloric intake in relation to body size of the growing individual of this intake carbohydrates supply a rather constant portion

In the patients with GGM the results of the carbohydrate tolerance tests have demonstrated a relationship between the removal rate of glucose from the blood and the dietary supply of well absorbed carbohydrates A low glucose tolerance is thus found when the diet contains very little fructose (case 1) Nevertheless a patient with GGM is capable of increasing the rate of removal of glucose from the blood when the intermediate metabolism has adapted to a larger supply of carbohydrates as demonstrated by the results of the i v GTT's in case 2

It may be concluded that patients with GGM show the same pattern of reaction on variations

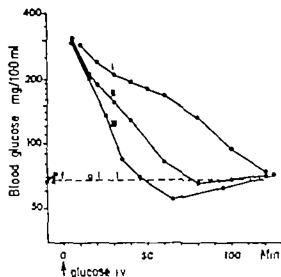


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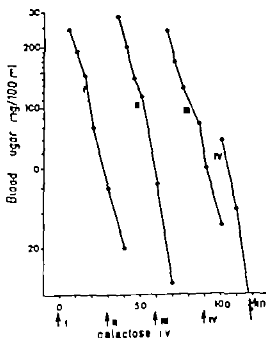


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[1] This infusion which augmented the blood sugar concentration up to levels of 900 mg/100 ml was given for measurement of the renal tubular reabsorption of glucose

malnourished signs of decreased glucose tolerance were seen with an oral fructose tolerance test In him as well as in the patient with glucose galactose malabsorption the supranormal rise of blood glucose was abolished by dietary measures ensuring an ample supply of calories and carbohydrates

The intravenous galactose tolerance tested in one of the patients was probably normal at 13 months of age and certainly normal at 6 years of age

It is concluded that the observed abnormalities of the metabolism of glucose in patients with glucose galactose malabsorption are unspecific for this disease and merely secondary to carbohydrate deprivation

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REFERENCES

1 Abraham J M Levin B Oberholzer V G & Russel A Glucose galactose malabsorption *Arch Dis Child 42* 59? 1967
Bowie M D Mulligan P B & Schwartz, R Intravenous glucose tolerance in the newborn infant the effects of a double dose of glucose and insulin *Pediatrics 31* 590 1963
3 Craig J W Miller M Macketune M S & Woodward H Jr The influence of dietary carbohydrate deprivation on the metabolism of intravenous administered fructose and glucose in man *J Clin Invest 37* 118 1958
4 Dahlqvist A Determination of maltase and isomaltase activities with a glucose-oxidase reagent *Biochem J 80* 547 1961
5 Dawes, G S & Shelley H J Physiological aspects of carbohydrate metabolism in the foetus and newborn in F Dickens P J Randle and W J Whelan (eds.) *Carbohydrate metabolism and its disorders* Academic Press London and New York 1968 vol pp 101 103
6 Donnel G N Wan G Ng Hodgman J E & Bergren W R Galactose metabolism in the newborn infant *Ped iatrics 39* 829 1967
7 von Euler U Larsson, Y & Persson B Glucose tolerance in the neonatal period and during the first m months of life *Arch Dis Child 39* 393 1964
8 Froesch, E R Prader A Labhart A, Stuber H W & Wolf H P Die hereditäre Fructoseintoleranz, eine bisher nicht bekannte kongenitale Stoffwechselstörung *Schweiz Med Wschr 87* 1168 195
9 Haverback B J Dyce B J Gutentag P J & Montgomery D W Measurement of trypsin and chymotrypsin in stool A diagnostic test for pancreatic exocrine insufficiency *Gastroenterology 44* 588 1963
10 Hevrovsky A A new method for the determination of inulin in plasma and urine *Clin Chim Acta 1* 470 1956
11 Kabi Laboratory Instructions Address KABI Fack S 124 25 Stockholm Sweden
12 Lindquist B Blockage of active transport of glucose *Transactions XI Int Congr Pediatrics Group panel discussions* p 85 Tokyo 1965
13 Lindquist B & Meeuwisse G W Intestinal transport of monosaccharides in generalized and selective malabsorption *Acta Paediat Scand Suppl 146* 110 1963
14 Loeb H Variations in glucose tolerance during infancy and childhood *J Pediat 68* 237 1966
15 Meeuwisse G W & Melin K Glucose galactose malabsorption A clinical study of 6 cases *Acta Paediat Scand Suppl 188* 3 1969
16 Orsini M & Stopa, T Comportamento della glicemia fruttosemia e glucosemia dopo carico orale di fruttosio e saccarosio nel bambino normale *Arch Ital Pediat 15* 122 1951
17 Oxman S V Maccioni A S Zuniga A C Spada, R G & Monckeberg F B Disturbances of carbohydrate metabolism in infantile marasmus *Am J Clin Nutr 21* 1285 1968
18 Persson B Sterky G & Thorell J Effect of low carbohydrate diet on plasma glucose free fatty acids glycerol ketones and insulin during glucose tolerance tests in adolescent boys *Metabolism 16* 714 1967
19 Ralander A Intravenous galactose tolerance test Normal series of children *Scand J Clin Lab Invest 2?* 196 1968
20 Somogyi M Determination of blood sugar *J Biol Chem 160* 69 1945
21 Tengstrom B An intravenous galactose tolerance test with an enzymatic determination of galactose a comparison with other diagnostic aids in hepatobiliary diseases *Scand J Clin Lab Invest 18 Suppl 92* 132 1966
22 Vink C L J Liver function and age *Clin Chim Acta 4* 674 1959

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in the intake of absorbable carbohydrates as do normal children from the newborn period and onwards

In case 2 at 13 months of age the removal rate of intravenously administered galactose was slightly retarded as compared to the two controls This is at variance with the finding of a normal elimination rate of galactose in an infant with GGM reported by Abraham *et al* (1) Although not subjected to investigations aimed at the detection of subclinical damage there were no signs of damage of the liver parenchyma in our patient The observation that orally given fructose which is well absorbed in patients with GGM produced values of the blood fructose concentration not exceeding 10–15 mg/100 ml does not support a suspicion of liver damage in that case higher blood fructose levels would have been expected Probably the half life of galactose of 11 min at 13 months of age is within the normal range of variation, despite the upper limit of 10 min found by Relander (19) He used a galactose dose of 0 35 g galactose per kg body weight which is about half the dose used here in children between 10 and 19 months of age A comparison of the few publications on galactose tolerance tests in children (6 19 22) indicates a relationship between dosage and the resulting half life values From these publications and preliminary studies in our department it seems that higher galactose doses produce larger half life values

In normal individuals oral administration of fructose results in a very modest rise or sometimes even in a slight fall in the blood glucose concentration (8 16) Hence although very helpful in the clinical diagnosis of GGM the observed rise in blood glucose concentration after the oral administration of fructose to patients with this disorder (15) cannot be regarded as a normal response to a FTT Nor is this finding specific for GGM the patient with cystic fibrosis (case 3) was found to react in the same way In this patient however the rise of blood glucose was perhaps not only due to decreased absorption of dietary CHO caused by disturbed digestion of starch but also to the low total supply of calories In both cases a normalization of the FTT was seen when the diet contained more absorbable CHO These observations are in agreement with experimental findings in adults subjected to CHO deprivation (3) the removal rate of intravenously injected fructose was not affected by the diet, but the resulting rise of blood glucose was found to be higher and more prolonged after several days of fasting or CHO deprivation than after a normal diet

The studies presented here indicate that patients with GGM possess a normal ability to adapt their glucose metabolism to varying intakes of absorbable carbohydrates and that observed signs of a reduced rate in glucose metabolism are secondary to dietary factors As the glucose metabolism normalizes on increasing amounts of fructose in the diet it is unlikely that in GGM the entrance of glucose into the liver and the peripheral cells is disturbed in a way similar to the entrance into the epithelium of the small intestine

SUMMARY

In 2 children with glucose galactose malabsorption the intermediate metabolism of hexoses was studied The removal rate of injected glucose was slow when the diet of the children contained limited amounts of well absorbed carbohydrate (fructose) When sufficient fructose was given in the diet, the half life of injected glucose was normal A fast removal rate was seen when large amounts of carbohydrates had been administered prior to the intravenous GTT The results of oral fructose tolerance tests varied with the pre test diet in a similar manner

The fructosemia was small and independent of the diet but the blood glucose concentration reacted in a way indicating decreased glucose tolerance if the diet contained little absorbable carbohydrate This was evidently an unspecific effect of carbohydrate deprivation in a control case an infant with cystic fibrosis initially

Table 1 *Excretion of cystathionine and catecholamines in twenty infants and children with neuroblastoma*
The values are expressed in mg per g creatinine n d = not determined

Case no	Sex	Age (years)	Cystathionine in urine	3 Methoxy-4-hydroxy mandelic acid in urine	Methoxy catecholamines in urine	Metastasis
1	♂	1/12	140	230 0	13.5	Yes
2		3/12	250	121 7	9 1	Yes
3	♂	4/12	0	43 5	17 9	Yes
4	♂	6/12	230	171 0	51 2	Yes
5	♀	6/12	180	32.2	5 9	No
6	♂	1	140	199 0	n d	Yes
7	♂	1	0	25 6	2 0	No
8		1 3/12	0	37 2	5 7	Yes
9		1 4/12	100	44 3	4 7	No
10	♂	1 6/12	160	36 3	n d	No
11	♂	2	0	28 7	n d	Yes
12	♀	2 6/12	260	90 3	19 5	No
13	♀	6 2/12	40	71 3	3 1	Yes
14	♂	3	40	29 7	7 2	Unknown
15	♀	4	90	44 0	9 8	Yes
16	♀	4	0	26 5	8 0	No
17	♂	4 6/12	20	23 5	5 8	Yes
18	♀	6	220	769 2	80 1	Yes
19	♀	6	0	75 3	19 8	Unknown
0	♂	10 6/12	100	107 3	8 9	Yes

logical amounts of 3 methoxy-4 hydroxymandelic acid and for methoxycatecholamines in urine These values are also given in the table

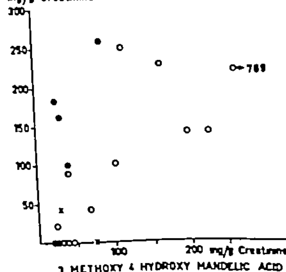


Fig 1 Relation hip between urinary excretion of cystathionine and 3-methoxy-4-hydroxymandelic acid in 0 children with neuroblastoma with metastases ( ) and without meta tasis ( ). = No record available about metasta is.

The figures in the table suggest a somewhat higher degree of cystathioninuria in children below the age of one year There seems to exist a relationship between the grade of catecholamine production and the presence of metastasis Because all 6 patients with a urinary excretion of 3 methoxy-4 hydroxymandelic acid greater than 100 mg per g creatinine had metastasis (Fig 1)

COMMENTS

The present investigation confirms the earlier reports of cystathioninuria in children with neuroblastoma Since the publication of an increased number of patients with neuroblastoma the supposed relationship (2 10) between cystathioninuria and the presence of metastasis has been found not to hold It appears now that only 2 of 3 cases with known metastasis exhibited cystathioninuria This figure may be somewhat less reliable because it is difficult to know for certain if metastasis are present or not On the other hand children with widespread metastases had no cystathioninuria All 20 patients with neuroblastoma of the material

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CYSTATHIONINURIA IN CHILDREN WITH NEUROBLASTOMA WITH AND WITHOUT METASTASIS

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Increased urinary excretion of the amino acid cystathionine has been observed A certain syndrome has been recognized which is called primary cystathioninuria It is an extremely rare inherited condition often combined with mental retardation and very probably caused by lack of activity of the cystathionine cleaving enzyme, cystathioninase (1)

Children with pyridoxine deficiency and diseases with gross hepatic failure have also been reported to excrete cystathionine in pathological amounts In this group of secondary cystathioninuria lack or increased need of pyridoxine has been held responsible for the increased excretion of this amino acid A third group of children with cystathioninuria has been described This group consists of children with malignant tumours of the sympathic nervous tissue (2 7 9 10) or with hepatoblastoma (4 13) Cystathioninuria in children with neuroblastoma was first described by Gjessing in 1963 This finding was confirmed in our laboratory (10) and by Shaw *et al* (9) Gjessing's and our impression was that in these children there is a correlation between cystathioninuria and the presence of metastasis or the degree of malignancy of the tumour The present investigation was undertaken to reevaluate the problem of metastasis and cystathioninuria based on a larger number of cases

MATERIAL

The material consisted of random collections of urine from 20 patients (12 boys and 8 girls) with histologically verified neuroblastoma The pattern of urinary excretion of methoxycatecholamines of this material has been published previously (11) The patients ranged in age from 1 month to 6 years These urine samples originate from children of various hospitals and were collected during a 3 year period The samples were acidified (pH 3) and had been stored at −20 up to 10 months before analysis

METHODS

The urinary amino acids were separated by high voltage paper electrophoresis combined with paper chromatography and cystathionine was measured as described before (12) Urinary methoxycatecholamines (metadrenaline and metanoradrenaline) were measured as described by Pisano (5) Urinary 3 methoxy 4 hydroxymandelic was determined as described by Pisano *et al* (6)

RESULTS

Fourteen of 20 children with neuroblastoma excreted increased amounts of cystathionine The amounts excreted varied between 20 and 260 mg per g of creatinine (Table 1) In 4 of these 14 patients no metastasis could be detected at the time of collection of urine and in one patient the patients records contained no note about any metastasis Six children with neuroblastoma exhibited no cystathioninuria Of these children 3 had metastasis and in 2 no metastasis could be detected clinically In one patient without cystathioninuria the patients records contained no note about any metastasis All children exhibited increased catecholamine production, as reflected in the patho

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CLINICAL AND BIOCHEMICAL GENETIC ASPECTS OF INTERMITTENT BRANCHED CHAIN KETOACIDURIA

Report of Two Scandinavian Families

H W GOEDDE U LANGENBECK, D BRACKERTZ W KELLER, T KOKKONES S HALVORSEN RAGNHILD KJIL and BRITA MERTON

From the Institute of Human Genetics University of Hamburg Hamburg Germany and the Paediatric Research Institute Rikshospitalet Oslo Norway

Since Pauling s description of sickle cell anaemia as a molecular disea e in 1949 the study of haemoglobin anomalies has given an impressive picture of geneti ally determined variation and has made possible the recogni tion of the relationship between molecular de fects and respective clinical disorders (13)

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In this paper we describe *observations* of Maple Syrup Urine Disease (*MSUD*) in two Scandinavian families who were affected with a variant of MSUD the intermittent type of MSUD (2 4 8 11) These forms can be clearly di tingui hed biochemically and clini cally from the so-called classical type first de scribed by Menke in 1954 (10) The geneti cally determined enzyme defect in classic MSUD is shown in Fig 1

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SUMMARY

Cystathioninuria was demonstrated in 14 of 20 children (aged 1 month to 6 year) with neuroblastoma associated with increased catecholamine production In 4 of these 14 patients no metastasis could be detected Two of the 6 children without cystathioninuria had metastasis Cystathioninuria appeared to occur more frequent in children with neuroblastoma in the age below 1 year Cystathioninuria in neuroblastoma is not an early sign of the disease and is not a reliable sign of metastasis

ACKNOWLEDGEMENT

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REFERENCES

1 Frimpter G W Cystathioninuria in J B Stanbury J B Wyngaarden and D S Fredrikson (eds) *The metabolic basis of inherited disease* McGraw Hill New York 1966 pp 409–419
2 Gjessing L R Studies of functional tumours II Cystathioninuria *Scand J Clin Lab Invest 15* 474 1963
3 — Studies of functional neural tumours III Cystathionine in the tumour tissue *Scand J Clin Lab Invest 15* 479 1963
4 Gjessing L R & Mauritzen K Cystathioninuria in hepatoblastoma *Scand J Clin Lab Invest 17* 513 1965
5 Pisano J J A simple analysis for normetanephrine in urine *Clin Chim Acta 5* 406 1960
6 Pisano J J Crout J R & Abraham D Determination of 3 methoxy 4 hydroxymandelic acid in urine *Clin Chim Acta 7* 285 1962
7 Raine D N Cystathioninuria in the diagnosis of abdominal tumours Abstract *Clin Chem 14* 786 1968
8 Scriver C R & Hutchison J H The vitamin B deficiency syndrome in human infancy Biochemical and clinical observations *Pediatrics 31* 240 1963
9 Shaw K N F Lieberman E Koch R & Donnell G N Cystathioninuria *Amer J Dis Child 113* 119 1967
10 v Studnitz Sulfur containing amino acids in the urine of patients with tumours from sympathic nervous tissue *Scand J Clin Lab Invest 17* Suppl *86* 190 1965
11 — Urinary excretion of methoxycatecholamines in neuroblastoma *Scand J Clin Lab Invest 21* 333 1968
12 — Secondary cystathioninuria *Acta Paediat Scand 58* 173 1969
13 Voute P A & Wadman S K Cystathioninuria in hepatoblastoma *Clin Chim Acta 22* 373 1968

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Cystathioninuria was demonstrated in 14 of 20 children (aged 1 month to 6 years) with neuroblastoma associated with increased catecholamine production. In 4 of these 14 patients no metastasis could be detected. Two of the 6 children without cystathioninuria had metastasis. Cystathioninuria appeared to occur more frequent in children with neuroblastoma in the age below 1 year. Cystathioninuria in neuroblastoma is not an early sign of the disease and is not a reliable sign of metastasis.

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REFERENCES

1. Frimpter, G. W.: Cystathioninuria. In J. B. Stanbury, J. B. Wyngaarden and D. S. Fredrikson (eds.): *The metabolic basis of inherited disease*. McGraw Hill, New York 1966, pp. 409–419.
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4. Gjessing, L. R. & Mauritzen, K.: Cystathioninuria in hepatoblastoma. *Scand J Clin Lab Invest 17*: 513, 1965.
5. Pisano, J. J.: A simple analysis for normetanephrine in urine. *Clin Chim Acta 5*: 406, 1960.
6. Pisano, J. J., Crout, J. R. & Abraham, D.: Determination of 3-methoxy-4-hydroxymandelic acid in urine. *Clin Chim Acta 7*: 285, 1962.
7. Raine, D. N.: Cystathioninuria in the diagnosis of abdominal tumours. Abstract. *Clin Chem 14*: 786, 1968.
8. Scriver, C. R. & Hutchison, J. H.: The vitamin B6 deficiency syndrome in human infancy. Biochemical and clinical observations. *Pediatrics 31*: 240, 1963.
9. Shaw, K. N. F., Lieberman, E., Koch, R. & Donnell, G. N.: Cystathioninuria. *Amer J Dis Child 113*: 119, 1967.
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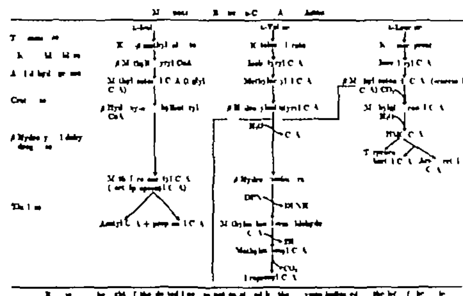


Fig 1 Metabolism of the branched chain amino acids The block in MSUD is localised in the second step of degradation

hospital with a 3 day history of bilateral purulent otitis media The first day she had fever (39 C) the second day she was afebrile but then she got a series of brief tonic convulsions and became progressively lethargic On admission she was afebrile semicomatose and had a strong curry odor She had a metabolic acidosis with total CO 7 mEq/l Urinary chromatography revealed excessive excretion of leucine and valine She remained unconscious for three days and had during this time several attacks of tonic and clonic convulsions with torsion spasms of the extremities

The convulsions were treated with luminal and chloral She gradually responded to intravenous infusion of electrolytes and glucose and was discharged well

The past history has been noncontributory She has had several acute infections without neurologic symptoms At present she is 7 / years old and of normal weight height and intellectual development The amino acid levels in serum and urine have been examined by chromatography on several occasions and have always been normal The mother has been told to restrict the intake of milk and to withdraw milk during periods of fever

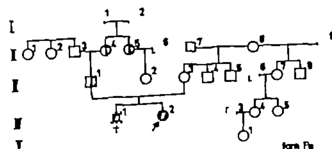


Fig 2 Pedigree of the family Fa first described in 1964 (4 8)

Symbols used in Figs 2 and 3 not studied □ studied normal enzyme activity ◫ studied enzyme activity about 50 of normal ◪ patients

B Fa (II 1) born March 1955 the 6 year-older brother of the preceding case He was admitted to the hospital 8 month of age because of otitis media fever vomiting and diarrhoea Except the vomiting there were no other neurologic symptoms or signs Apart from this one episode and a few periods in wintertime of acute bronchitis he was healthy and developed well physically and mentally In school he was considered one of the brightest boys in the class In Jan 1963 he was again admitted to the hospital under suspicion vomiting and lethargy On the admission he was afebrile semicomatose with high pitched cries and with frequent torsion spasms of the extremities He had metabolic acidosis with total CO 9 mEq/l There was a strong curry like smell of the urine reminding of the odor of the sister when she was comatose 4 month previously

Despite intravenous infusion of fluids and electrolytes he lapsed into coma He responded poorly to anticonvulsive treatment Because of respiratory failure he was intubated and put on artificial respiration and later a tracheotomy was performed In addition there was a cardiovascular failure and he was kept on Aramine the last two days He died 5 days after admission

By chromatography of urine and serum there was found an excessive excretion of valine and leucine/isoleucine and a great elevation of the same amino acids in serum Excessive amount of α ketoisocaproic acid (from leucine) and/or α keto β methylvaleric acid (from isoleucine) were found in urine

Autopsy revealed spongy degeneration of the deep layers of cortex and necrosis of the granule cell layer of the cerebellum There was also a considerable nerve cell loss in the pontine nuclei and in substantia nigra

The other members of the family

There has been no anamnestic clue of early death of other children in the family nor of neurologic attacks of any kind

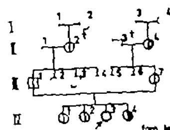


Fig. 3. Pedigree of the family Ja.

Family Ja (*Pedigree Fig. 3*)

Case 1. Ja (*II 3*), a girl born September 1964 as the third of four sisters of unrelated parents. The child was born at term after a normal pregnancy and has developed normally. She has been healthy except for the two episodes described in the following. At 1 year she had an episode of fever when she became soporous. The mother observed a peculiar smell from the napkins.

At the age of two year she got an upper respiratory infection and had a temperature between 39 and 40 C for two days. The following day she was febrile but refused to eat and had periods of unconsciousness. She lay in opisthotonus and was referred to the Paediatric Department, Rikshospitalet, Oslo with the diagnosis of meningitis. On admission she was cerebrally affected and without contact. The deep tendon reflexes were absent. She reacted to pain but not to other stimuli.

Temperature 36.4 C. The cerebrospinal fluid was normal for cells, protein and sugar. Blood leucocytes were 19 900/mm, blood sugar 37 mg/100 ml, blood pH 7.27, pCO 24 mm Hg and standard bicarbonate 14 mEq/l. The urine had strong curry-like smell suggestive of maple syrup urine disease and gave a markedly positive reaction with 2,4-dinitrophenyl hydrazine. Amino acid chromatograms showed increased amount of leucine, isoleucine and valine both in urine and serum and this was confirmed by quantitative determination on a Technicon amino acid analyzer. EEG the day after admission showed marked generalized dysrhythmia, indicating diffuse organic cerebral involvement. She was treated with ACTH and intravenous fluids. Attempts to give her a synthetic diet low in the branched chain amino acids were not successful and she was therefore given a milk free diet. She rapidly improved from the second day onwards. EEGs taken on the fourth day of hospitalization showed still marked dysrhythmia, on the 11th day normalization and on the 16th day it was normal. Amino acid chromatograms of urine and serum showed normalization after a few days and were normal one week after admission. She is now 5 years old and has had no other episodes of the disease. She is without neurological and mental symptoms. Psychological examination has shown that her mental development is above average for age.

BIOCHEMICAL GENETIC INVESTIGATIONS

Methods

The activities of branched-chain keto acid oxidases were estimated in the leukocytes of the peripheral blood. Substrates were 1-C labelled branched chain alpha keto acids: alpha ketoisocaproic acid (KIC), alpha keto isovaleric acid (KIVA) and alpha keto beta methyl valeric acid (MEVA). The CO produced was measured. Enzyme activities are dealt with as mμmoles ketoacid converted to CO by 16 million leukocytes in 3 hours at 37 C (3). The number of lymphocytes in the white blood cell suspensions was determined in order to judge the enzyme activities more precisely for reasons described previously (7). Activities obtained from normal homozygotes for the classic form of MSUD are summarized in Table 1 (6).

RESULTS

Family Fa was investigated in 1967 and 1968 and Fam. Ja in 1968. The results of these investigations can be seen in Figs. 2 and 3. In each family one of the parents (in family Fa mother III 3 and in family Ja father III 1) and all tested relatives of this particular parent showed normal enzyme activities in our heterozygote test. In contrast the father III 1 of the family Fa and the mother III 7 of family

Table 1. Conversion of branched chain ketoacids with enzymes from leukocytes of normal homozygous and heterozygous persons

Oxoacid	Converted substrate (mμ moles/16 · 10 leucocytes)					
	Normal homozygotes			Heterozygotes		
	n	Mean	Standard deviation	n	Mean	Standard deviation
α-oxo-isocaproic acid	71	3.25	0.72	39	1.47	0.46
α-oxo-isovaleric acid	32	4.04	0.78	79	1.79	0.61
α-oxo-β-methylvaleric acid	7	3.09	0.78	27	1.44	0.34

Table 2 *Conversion of branched chain ketoacids with enzymes from leukocytes of patients with intermittent maple syrup urine disease*

		Converted substrate (mμ mol s/ 1 6 × 10⁷ leukocytes)		
		KIC	KIVA	MEVA
Fa IV 2	August 1967	0 98	1 38	0 24
	August 1967	0 58	1 61	1 01
	April 1968	0 07	0 68	0 26
Ja IV 3	April 1968	0	0	0

Ja had enzyme activities in the range of 50 per cent of normal value Similar values were observed in some relatives of these two parents Results of activity measurements in the two patients are given in Table 2 Patient Fa IV 2 displayed minimal activity in peripheral blood cells The test has been repeated 9 month later with the same result The conversion rate of KIC was the lowest of the three substrates With our method patient Ja IV 3 showed no enzyme activity at all

DISCUSSION

Biochemical genetic findings suggest two differences between the classical and intermittent type of MSUD

1 In the classical form both parents have decreased enzyme activities (about half the amount of normals) whereas in the intermittent form only one parent had decreased activity of the branched chain ketoacid oxidases This phenotype is inherited as the heterozygous manifestation of an autosomal gene The other parent and his relatives showed all normal activity values in the heterozygote test

2 Patients with the intermittent form sometimes or always have residual activities of the respective oxidases in peripheral blood cells (the turnover of the leucine derivative KIC is lowered the most) whereas in classical MSUD the atypical homozygous patients have no activity at all

For interpretation of 1 Only standard activity measurements have been performed and therefore nothing can be concluded about the underlying biochemical alterations in enzyme protein

It is not clear whether parents with diminished enzyme activity are heterozygous for the allele of classic MSUD There are several ways *to explain normal activity in either parent*

(*a*) Homozygosity for the normal allele but this seems to be highly improbable

(*b*) *Parents with normal activity may* be heterozygous for an allele controlling the synthesis of an enzyme with only minor alteration in substrate affinity Similar observations have been made in some families with pyruvate kinase deficiency where small alterations in substrate affinity have been observed (12) *so-called dominant inheritance is not to be as*sumed

(*c*) The possibility exists that parents with normal activity may have an allele producing a polypeptide chain which combines with a normal gene product to form a normally functioning enzyme whereas in doubly heterozygous persons the patients a combination may occur resulting in synthesis of an enzyme with grossly impaired function leading to the clinical symptoms of MSUD In *complementation studies* on bacteria and moulds such enzymes with normal activity have been *found to be unstable* in heat denaturation experiments (15)

For interpretation of 2 Connelly and coworkers *consider* the classic MSUD to be a defect only of KIC and MEVA oxidation both of which they assume to be catalysed by one single enzyme (3)

We have found in heterozygotes for classic MSUD a 50 per cent diminution of the activities of all three oxidase complexes of the catabolism of valine leucine and isoleucine (Table 1) (6)

These results and our findings of a different distribution of KIC and *MEVA oxidase* activities in peripheral blood (7) are not in accordance with the existence of a single KIC MEVA *oxidase as proposed* by Connelly *et al* Observations in another child with intermittent MSUD (11) seem to point to an isolated defect of KIC oxidation in intermittent MSUD

Diminution of the KIVA and MEVA-oxida tion in such cases may be caused by competi tive inhibition However as yet we have no information on the size of the intracel lular pool of ketoacids in classic and intermit tent MSUD

The failure to show any activity in leuko-cytes of the patient in family Ja during a period free of symptoms may be owing to technical difficulties because of a low yield of $^{14}CO_2$ Another explanation at this time is not possible

Mechanisms leading to acidotic attacks are of special interest La Du (9) has shown that according to the special kinetic properties of phenylalanine hydroxylase an intermittent course of hyperphenylalaninemia is to be ex pected if the mutant enzyme has a diminish d affinity to the pteridine cofactor

Further studies on normal and mutant branched chain keto acid oxida e are needed to larify the respective mechani m in intermit tent MSUD

SUMMARY

Intermittent branched-chain ketoaciduria was detected in two Norwegian families All three patients had normal intelligence One died dur ing an acute acidotic attack at the age of 8 years

The biochemical and clinical data in the two families suggest a separate variant of branched chain ketoaciduria Possible mechanisms of ge netic heterogeneity in this disorder are dis cussed

REFERENCES

1 Ando T Nyhan W L Gerritsen T Gong L Heiner D C & Bray P F Metabolism of gly cine in the nonketotic form of hyperglycinemia P [illegible] R 54 1968

2 [illegible] e J Maple syrup urine disease Arch Fran Ped 5 601 1968

3 Bowde J A & Connelly J L Branched chain keto-acid metabolism II Evidence for the common identity of α keto isocaproic acid and α keto-β methyl valeric acid dehydrogenases J B l Chem 243 35 4 1969

4 Dancis J Hutzler J & Rokkones T Intermit tent branched-chain ketonuria Variant of maple syrup urine disease New Eng J Med 76 84 1967

5 Goedde H W Richter E Hufner M & Steel B Arbeitsvorschrift eines vereinfachten Hetero-zygotentestes fur die Ahornsirupkrankheit Klin Wschr 42 818 1964

6 Goedde H W & Keller W Metabolic pathways in maple syrup urine di ease In W L Nyhan (ed) Amino acid metabolism and genetic varia tion McGraw Hill Book Comp New York To-ronto and London 1967 p 191

7 Goedde H W Langenbeck U & Brackertz, D Detection of heterozygotes in maple syrup urine disease Role of lymphocyte count Hu mangenetik 6 189 1968

8 Kiil R & Rokkones T Late manifestin variant of branched-chain ketonuria (maple syrup urine disease) Acta Paediat Scand 53 356 1964

9 La Du B N Genetic variation in metabolic di orders In C L Nyhan (ed) Amino acid metabolism and genetic variation McGraw Hill Book Comp New York Toronto and London 196 p 121

10 Menkes J H Hurst P L & Craig, M New syndrome Progre sive familial infantile cerebral dysfunction associated with unusual urinary sub-stances Pediatrics 14 462 1954

11 Muller H & Bickel H To be published

12 Paglia D E Valentine W N Baughan M A Miller D R Reed C F & McIntyre O R An inherited molecular lesion of erythrocyte pyruvate kina e J Clin I est 47 1929 1968

13 Perutz M F & Lehmann H Molecular patho-logy of human haemoglobin Nature 219 902 1968

14 Rosenberg L E Genetic heterogeneity in Cys tinuria In W L Nyhan (ed) Amino acid meta bolism and genetic va iation McGraw Hill Book Comp New York Toronto and London 1967 p 341

15 Siddiqi O Interallelic complementation in vivo and in vitro Brit Med Bull 21 249 1965

16 Williams H E & Smith L H Jr L-glyceric aciduria A new genetic variant of primary hyperoxaluria New Eng J Med 78 233 1968

17 Nomenclature of Glucose 6 phosphate dehydro-genase in man WHO Techn Rep S 1967 366

18 Yoshida A A single amino acid substitution (asparagin e to aspartic acid) between normal (B+) and the common Negro variant (A+) of human glucose 6 phosphate dehydrogenase Proc Natl Acad Sci 57 835 1967

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Table 2 *Conversion of branched chain ketoacids with enzymes from leukocytes of patients with intermittent maple syrup urine disease*

		Converted substrate (m*μ* mol s/ 1 6 × 10⁷ leukocytes)		
		KIC	KIVA	MEVA
Fa IV 2	August 1967	0 98	1 38	0 24
	August 1967	0 58	1 61	1 01
	April 1968	0 07	0 68	0 26
Ja IV 3	April 1968	0	0	0

Ja had enzyme activities in the range of 50 per cent of normal value Similar values were observed in some relatives of these two parents Results of activity measurements in the two patients are given in Table 2 Patient Fa IV 2 displayed minimal activity in peripheral blood cells The test has been repeated 9 month later with the same result The conversion rate of KIC was the lowest of the three substrates With our method patient Ja IV 3 showed no enzyme activity at all

DISCUSSION

Biochemical genetic findings suggest two differences between the classical and intermittent type of MSUD

1 In the classical form both parents have decreased enzyme activities (about half the amount of normals) whereas in the intermittent form only one parent had decreased activity of the branched chain ketoacid oxidases

This phenotype is inherited as the heterozygous manifestation of an autosomal gene The other parent and his relatives showed all normal activity values in the heterozygote test

2 Patients with the intermittent form sometimes or always have residual activities of the respective oxidases in peripheral blood cells (the turnover of the leucine derivative KIC is lowered the most) whereas in classical MSUD the atypical homozygous patients have no activity at all

For interpretation of 1 Only standard activity measurements have been performed and therefore nothing can be concluded about the underlying biochemical alterations in enzyme protein

It is not clear whether parents with diminished enzyme activity are heterozygous for the allele of classic MSUD There are several ways to explain normal activity in either parent

(*a*) Homozygosity for the normal allele but this seems to be highly improbable

(*b*) Parents with normal activity may be heterozygous for an allele controlling the synthesis of an enzyme with only minor alteration in substrate affinity Similar observations have been made in some families with pyruvate kinase deficiency where small alterations in substrate affinity have been observed (12) so called dominant inheritance is not to be assumed

(*c*) The possibility exists that parents with normal activity may have an allele producing a polypeptide chain which combines with a normal gene product to form a normally functioning enzyme whereas in doubly heterozygous persons the patients a combination may occur resulting in synthesis of an enzyme with grossly impaired function leading to the clinical symptoms of MSUD In complementation studies on bacteria and moulds such enzymes with normal activity have been found to be unstable in heat denaturation experiments (15)

For interpretation of 2 Connelly and coworkers consider the classic MSUD to be a defect only of KIC and MEVA oxidation both of which they assume to be catalysed by one single enzyme (3)

We have found in heterozygotes for classic MSUD a 50 per cent diminution of the activities of all three oxidase complexes of the catabolism of valine leucine and isoleucine (Table 1) (6)

These results and our finding of a different distribution of KIC and MEVA oxidase activities in peripheral blood (7) are not in accordance with the existence of a single KIC MEVA oxidase as proposed by Connelly *et al* Observations in another child with intermittent MSUD (11) seem to point to an isolated defect of KIC oxidation in intermittent MSUD

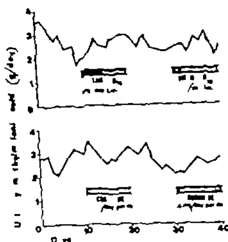


Fig. 1. Rate of urinary excretion of methylmalonic acid before and after treatment with 1 or 2.5 mg/day i.m. of vitamin B_{12} and 2 or 4 mg/day per os of cobamide coenzyme.

supplied by Recordati S.A.S., Milano, Italy) was also ineffective (see Fig. 1).

The metabolic block in the methylmalonic acidemia is in the two step conversion of methylmalonyl CoA to succinyl CoA (5). In the first step the D-methylmalonyl CoA is converted into its isomer by the enzyme methylmalonyl CoA racemase, in the second step the L-methylmalonyl CoA is transformed into succinyl CoA by methylmalonyl CoA mutase together with the cobamide coenzyme. There are no specific studies determining the precise site of the defect. However, the decreased excretion of MMA after vitamin B_{12} or cobamide coenzyme administration suggests that the block is in the L-methylmalonyl CoA→succinyl CoA reaction.

Nevertheless our observation points out the existence of a form of methylmalonic acidemia unresponsive to vitamin B_{12}.

Therefore we are doubtful that methylmalonic acidemia can be simply considered a state of vitamin B_{12} dependency and we think that there may be different genotypes of the disease.

This is in agreement with Auerbach's *et al.* conclusions based on the enzymatic studies of liver specimens of four patients with methylmalonic acidemia. They observed that none of the liver extracts of those four patients produced succinate from methylmalonate in the absence of vitamin B_{12} and only one could normally form succinate in the presence of vitamin B_{12}.

When a substantial number of patients will have been studied it will be possible to determine which are the clinical and biochemical differences between the vitamin B_{12} responsive and unresponsive variants of methylmalonic acidemia.

REFERENCES

1. Auerbach, V. H., Morrow, G., Di George, A. M. & Barness, A. L.: Methylmalonic acidemia and glycinemia: various genotypes with enzymatic studies. Abstracts Federation of European Biochemical Societies. Sixth Meeting, Madrid 7–11.4.1969, p. 282.
2. Lindblad, B., Lindstrand, K., Svanberg, B. & Zetterstrom, R.: The effect of cobamide coenzyme in methylmalonic acidemia. *Acta Paediat Scand 58*: 178, 1969.
3. Oberholzer, V. G., Levin, B., Burgess, E. A. & Young, W. F.: Methylmalonic aciduria. An inborn error of metabolism leading to chronic metabolic acidosis. *Arch Dis Child 42*: 492, 1967.
4. Piez, K. A. & Morris, L.: A modified procedure for the automatic analysis of amino acids. *Anal Biochem 1*: 187, 1960.
5. Rosenberg, L. E., Lilljeqvist, A. Ch. & Hsia, Y. E.: Methylmalonic aciduria: metabolic block localization and vitamin B_{12} dependency. *Science 162*: 805, 1968.
6. Stokke, O., Eldjarn, L., Norum, K. R., Steen-Johnsen, J. & Halvorsen, S.: Methylmalonic acidemia. A new inborn error of metabolism which may cause fatal acidosis in the neonatal period. *Scand J Clin Lab Invest 20*: 313, 1967.

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SHORT COMMUNICATION

METHYLMALONIC ACIDEMIA AND VITAMIN B_{12} DEPENDENCY

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In a patient with methylmalonic acidemia, a recently described inborn error of metabolism (3, 6), Rosenberg *et al.* (5) have reported a marked decrease of urinary excretion of methylmalonic acid (MMA) after daily parenteral administration of 1 mg of vitamin B_{12}. In two other cases Lindblad *et al.* (2) have found that MMA excretion during a tolerance test with valine was more than half diminished when 5 mg of cobamide coenzyme was given before the test.

Recently we have had the opportunity of giving large doses of vitamin B_{12} and cobamide coenzyme to a child with methylmalonic acidemia. However we did not observe a decrease in the excretion of MMA.

B. G., a boy, was hospitalized the first time at birth in December 1965 because his three brothers had died between the third and fifth days of life of an unknown cause. On the fourth day of life the patient had a severe metabolic acidosis crisis with ketosis and recovered with parenterally infused fluids: sodium bicarbonate and glucose. During the next three years the child had 8 other severe episodes of ketoacidosis, often during an infection. In the intervals the child is clinically healthy and has only a mild compensated metabolic acidosis without ketonuria.

The infant has always been difficult to feed and refuses foods rich in proteins. His diet, supplemented daily with 2 g of sodium bicarbonate, has never had a protein content higher than 2.5 g/kg. Physical and mental development is retarded. At 3 years of age the boy's weight was 11 kg, height 88 cm and his I.Q. 88. The liver and spleen are not enlarged. The lungs, heart, nervous system and muscle tone are normal. The MMA has been identified (for the first time in July 1968) by paper chromatography and determined according to Oberholzer *et al.* (3). In the serum MMA ranged from 5 to 33 mg/100 ml and in the urine from 0.8 to 4.9 g/day. The plasma glycine concentrations determined with an amino acid analyzer according to Piez & Morris (4) were 2.9 and 5.3 mg/100 ml during a ketoacidosis crisis and 9.6 and 13.5 mg/100 ml during a satisfactory clinical period. Plasma lactate levels vary from 14 to 64 mg/100 ml and the serum uric acid ranges from 6.9 to 17.1 mg/100 ml.

Mild anemia (Hb 8.4–10.6 g/100 ml, Htc 27–35) with normal or a little higher reticulocyte counts (1.2–2.7 %) is usually present. During two acidosis crisis we observed a severe anemia (Hb 5.4 and 5.1) and in one of them erythrocyte ^{51}Cr halflife was 12 days.

Sometimes we observed neutropenia but not thrombocytopenia. Megaloblastic changes were not present in the bone marrow.

No modification in MMA excretion was observed when the patient placed on a constant diet (2.5 g protein per kg) was given 1 or 2.5 mg/t m daily of vitamin B_{12}. The administration of 2 or 4 mg per os daily of 5,6 dimethyl benzimidazole cobamide coenzyme (kindly

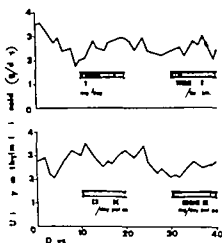


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REFERENCES

1 Auerbach V H Morrow G Di George A M & Barness A L Methylmalonic acidemia and glycinemia various genotypes with enzymatic studies *Abstracts* Federation of European Bio chemical Societies Sixth Meeting Madrid 7–11 4 1969 p 282

2 Lindblad B Lindstrand K Svanberg B & Zetterstrom R The effect of cobamide coen zyme in methylmalonic acidemia. *Acta Paediat Scand 58* 178 1969

3 Oberholzer V G Levin B Burgess E A & Young W F Methylmalonic aciduria An in born error of metabolism leading to chronic metabolic acidosis *Arch Dis Child 42* 492 1967

4 Piez, K A & Morris L A modified procedure for the automatic analysis of amino acids *Anal Biochem 1* 187 1960

5 Rosenberg L E Lilljeqvist A Ch & Hsia Y E Methylmalonic aciduria metabolic block localization and vitamin B dependency *Science 162* 805 1968

6 Stokke O Eldjarn L Norum K R Steen Johnsen J & Halvorsen S Methylmalonic acidemia A new inborn error of metabolism which may cause fatal acidosis in the neonatal period *Scand J Clin Lab Invest 20* 313 1967

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CASE REPORT

ACUTE LEUKAEMIA WITH MEDIASTINAL INFILTRATE SIMULATING PERICARDITIS

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Recently we had the opportunity to study a patient with a mediastinal leukaemic infiltration simulating pericarditis Because of the lack of information on this rather unusual clinical picture in the standardbooks of haematology we deemed it worthwhile to report this patient

CASE HISTORY

P T a 12 year old boy was referred to us on May 10 1966 A few days earlier he had been admitted to another hospital because of a bleeding tendency after a recent adeno tonsillectomy The consulting pediatrician diagnosed an acute leukaemia and a cardiomegalia and or pericarditis For further investigation he was sent to this hospital In 1965 he had been treated successfully for a pulmonary tuberculosis

On admission we found a sick boy in a good nutritional condition The pulse was 100 pm rather well filled bloodpressure was 100 60 mm Hg Respiratory movements were normal The praecordium was somewhat bulging Cardiac dullness extended from the right sternal border to the left anterior axillary line The cardiac sounds were very soft pericardial rubbing or intracardiac murmurs were absent The lungs were normal by auscultation and percussion

The liver reached 5 cm the spleen 4 cm below the costal border Lymphnodes in the axillae the groins and in the neck were small

Laboratory investigations ESR 13 mm Hb 13 2 g/100 ml thrombocytes 79 000/mm White cell count 250 000/mm (73 blast cells) The urine was normal The bonemarrowsmear (May Grunwald Giemsa stain) contained still normal cells of the erythroid and myeloid series The majority were abnormal they had a diameter of 20 to 30 μ with dense nuclei some of them containing 1 to 5 nucleoli The cytoplasm was dark blue with vacuoles but contained no granules About 30 of the blast cells were smaller with an average diameter of 10 μ In the blood smear these smaller cells were more numerous The diagnosis of acute leukemia probably of the myeloblastic type was made

X ray investigations on thorax X ray an enlarged heart contour was seen There was pleural fluid on the left side Old healed calcified tuberculous lung lesions were present

At fluoroscopy no cardiac pulsations were noted except at the lower right border The upper mediastinum seemed to be broadened but no scalloping was seen either of the upper mediastinum or of the cardiac contours

ECG normal sinusrhythm axis 60 PQ normal QT 0 28–0 29 sec (normal) The S T segments were somewhat rounded The T tops looked more normal than is found usually in a long standing pericarditis

Treatment and course The diagnosis acute leukemia was beyond all proof We had no clear cut idea about the pericarditis like picture That this of tuberculous origin was seemed improbable no signs of activity had been noted at any moment of follow up

We started treatment with vincristine rubidomycine and prednison and administered also antituberculous drugs Two days after starting treatment the patient developed a fever of unknown origin Blood cultures remained sterile Completely unexpectedly he died on May 13

Autopsy

At autopsy a large greyish white lobulated mass was observed within the anterior mediastinum This mass extending from the left anterior cervical lymph nodes down to the diafragm was 5 cm thick where covering the heart and had a total weight of 500 grams



Fig 1 Thorax X ray on admission

The tumor infiltrated the anterior wall of the p ri cardium It reached up to the mesothelium without breaking through *The heart was laying free in the pericardial* s and the cavity contained a normal amount of c ear yellow fluid The neoplasm encircl d the great es els within the m dia tinum

At microscopy the tumor cells were arrang d in solid nodular field di ided by ascular fibrous septa Two definite cell types could be re ognized

One type were large cells with a great round some what lobulated nucleus containing one or more nucleoli and a scarce pink cytop asm The chromatin was di crete and irregularly divided

The other type were small cells with a small round hyperchromatic nucleus and a scarce pink cytoplasm In these nuclei no nucleoli were found Staining for newly formed reticulin fibers were negative

I an extensive study of many parts of the whole ma no remnants of preexistent thymic structure ould be observed The cytological *picture of the* cells found in this mass su gests the same origin as the leukaemic cells that were noted in the bone mar *row and elsewhere*

The pathological diagnosis was Extensive tumor ous l ukaemic infiltration of the anterior medias tinum The regional lymph nodes were enlarged but nowhere grown together In addition leukaemic in filtrates were found in the heart liver spleen kid neys adrenals and testis

DISCUSSION

Cardiac manifestations in acute leukemia do not re eive great attention in the standard text books (3 10 11) The roentgenologic inves *tigations in this patient tempted us to make the* diagno i of pericarditis The electrocardio gram however did not favour this *diagnosis*

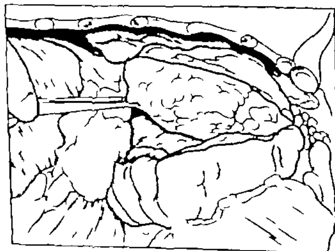


Fig 2 Pen drawing of the opened thorax at autopsy The leukaemic in filtrate is divided part of the anterior aspect of the pericardium is seen No adhesions were present between the in filtrate and the smooth pericardium

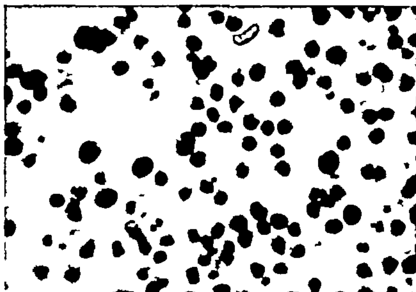


Fig 3 Histology of the infiltrate (H E Stain) Magnification 1600 two dif ferent cell types can be seen in this picture

Bierman *et al* (1) reported four patients with acute leukaemia who developed the clinical picture of pericarditis during the course of their disea e The electrocardiogram revealed tachycardia low voltage elevations of the S T segment and flat to inverted T waves Haddy (4) described a 3 year old girl with acute leu kaemia in which an enlarged heart was found with left pleural fluid A presumptive diagnosis of congestive heart failure due to leukaemic infiltration of the myocardium was made The heart and the electrocardiogram became nor mal after cardiac irradiation The usual treat ment of cardiac failure with digitalis and diure tics tried before irradiation had had no effect At autopsy 6 weeks after the clinical cure of the heart failure the epicardium was thickened but the pericardium and myocardium appeared normal

Klatte *et al* (7) report a eries of 341 pa tients with leukemia in which roentgenograms and clinical records could be studied Hilar and mediastinal enlargement was often seen with clear demarcation of the lymphnodes so that there was scalloping of the margins Only occasionally (5 cases) the mediastinal enlargement would be diffuse with smooth margins One patient with superior venacaval obstruc tion was seen Cardiac enlargement was noted often particularly in children with acute leu kemia In 35 5% autopsy was also performed

Specifically they do not mention the pres ence of large mediastinal tumours No special mention of it is made in the large clinical and pathological study of 123 cases of leukaemia of Kirschbaum & Preuss (5) Therefore it seemed very remarkable to us that the only report of similar cases in children dates back to 1932 Cooke described nine patients aged 3 to 13 years all boys with a mediastinal tumour Four had no leukaemic blood picture initially The mass and the eventual initial dyspnoea disap peared after irradiation In two patients au topsy was performed These showed a big tu mour mass extending from the thymic region over the pericardium The tumours consisted of fields of mononuclear cells held together by connective tissue stroma No thymic rests were found in these tumours We believe that these patients have very much in common with our patient

The mediastinal tumours are very sensitive to X ray (2) In our opinion it is not imposs ible that Haddy (4) actually reported a similar case that was treated succesfully with irradia tion Kho Lien Keng (6) described a 6 year old boy with signs of tracheal compression caused by a superior mediastinal tumour signs and symptoms disappeared after local irradiation Some weeks later the blood picture became leukaemic

Local irradiation seems to of value antileu-

kaemic drug therapy may probably have the same good effect

We cannot explain the sudden death of our patient Mathé *et al* (1968) mention the toxic effect of rubidomycin on the heart But neither Mathe (8) or Massimo *et al* (9) report sudden deaths as a result of rubidomycine therapy Rubidomycin was given by us to about 30 children without giving rise to cardiac complications

We are tempted to believe that the tumour suddenly gave rise to serious circulatory and/or respiratory failure A definite proof for this is however lacking

While the diagnostic procedures during life always are limited knowledge of the existence of such a clinical picture can lead to an early correct diagnosis

REFERENCES

1 Bierman H R Perkins E K & Ortega P Pericarditis in patients with leukemia *Amer Heart J 43* 413 1952

2 Cooke J V Mediastinal tumor in acute leukemia a clinical and roentgenological study *Amer J Dis Child 44* 1153 1932

3 Dameshek W & Gunz, F *Leukemia* Grune & Stratton New York and London 1964 2nd ed

4 Haddy T B Cardiac irradiation in childhood leukemia *Amer J Dis Child 108* 539 1964

5 Kirshbaum J D & Preuss F S Leukemia a clinical and pathological study of 123 fatal cases in a series of 14 400 necropsies *Arch Int Med 71* 777 1943

6 Kho Lien Keng *Nieuwe aspecten van diagnostiek en therapie van leukemieen bij kinderen* Thesis, Amsterdam 1952

7 Klatte E C Yardley J Smith E B Rohn R & Campbell J A The pulmonary manifestations and complications of leukemia *Amer J Roentgen 89* 598 1963

8 Massimo L Fossati Guglielmoni & Fortuna E Daunomycin in the treatment of acute leukemia and neoplastic diseases in childhood *Helv Paed Acta 23* 315 1968

9 Mathe G Hayat M Schwarzenberg L Amiel J L Schneider M Cattan A Schlumberger J R & Jasmin C Haute frequence et qualite des remissions de la leucémie aiguë lymphoblastique chez l'enfant induites par l'association de delta 1-cortisone leurocristine et rubidomycine *Arch Franç Ped 25* 181 1968

10 Smith D H *Blood diseases of infancy and childhood* C V Mosby Comp St Louis 1966 2nd ed

11 Wintrobe M N *Clinical Haematology* Lea & Febiger Philadelphia 1967 6th ed

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CASE RFPORT

HEREDITARY ECTODERMAL DYSPLASIA

Report of Two Cases

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Ectodermal dysplasia is usually characterized by the triad hypodontia hypotrichosis and hypo- or anhidrosis but other ectodermal manifestations are also een (1 2 12) Up to the year 1947 80 cases had been published (16) and by 1967 about 300 (9)

From Sweden Ihre (11) described a child with anodontia in combination with other ecto dermal stigmata In a clinical and genetical in vestigation Grahnen (7) studied the frequency of hypodontia in a Swedish population In thi serie of about 2000 individuals no typical case of ectodermal dysplasia was found From Nor way Rinvik & Syrrist (15) described one case of extreme hypodontia in connection with other ectodermal defects and Hasvold (8) two siblings with typical signs of ectodermal dysplasia From Denmark a number of ca e re ports have been published (9 10 12)

Awwad & Essawy (1) stressed the impor tance of being acquainted with the syndrome They particularly emphasized that infants with ectodermal dysplasia becau e they lack sweat gland can in high environmental temperature develop a feverish condition which can even result in death

In this paper two cases of ectodermal dys plasia are presented Both patients showed a very marked temperature instability

CASE REPORTS

Case P B a boy born September 22 1961 oldest of two children The parents and the younger child were healthy Wassermans reaction of the mother was negative Pregnancy delivery and neonatal per iod were uncomplicated Birth weight was 3770 g and height 51 cm The boy s maternal grandmother and great grandmother had a number of congenitally missing teeth and the grandmother h d al o noticed diminished perspiration Otherwise they and their re latives were healthy

The boy s physical and psychomotor development was normal The parents noted early that he was very ensitive to heat exposure During warm and sunny days his temperature ro e to 39 C without any signs of infection The mother noted that he could not perspire All the time he had severe nasal se retions and heavy crusting in the nose with a foul odour Furthermore at an age of he had only two erupted pee shaped central primary incisors At this age he was admitted to the Department of Paedi atrics at the County Hospital Lidkoping

The physical examination revealed a normally de veloped boy without any infectious symptoms His weight and height were within the normal limits His ears were bowl shaped and protruding The eyelashes and eyeb ows were anty The scalp hair was fine blond and sparse His nasal bridge was flat and his lip protruding The finger and toenails were normal Beneath the eyes he had bilateral linear wrinkles His skin was dry and he had minor eczematoid lesions on the forearm

Haemoglobin concentration was 11 8 g/100 ml WBC was 9700 Differential leukocyte count showed a normal distribution MSR 11 mm PBI 7 0 μg/100 ml Cholesterol 171 mg 100 ml The erum protein electrophoresis was normal and so was the quantita tive determination of immunoglobulins Cytogenetical

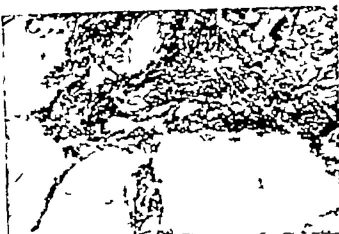


Fig 1 Section of skin (axillar region) from patient P B showing no sweat glands or sebaceous glands (× 40)

examination of peripheral leukocyte cultures showed normal chromosome constitution Urine examination for protein and glucose was negative

A roentgenographic examination of the jaws showed underdeveloped alveolar processes In the lower jaw there were no tooth buds and in the upper jaw only the primary central incisors and canines (03+ 01+ +01 and +03) and the permanent central incisors (1+ +1) were detected. Only two primary teeth had erupted (01+ and +01) and both of them were peg shaped The roentgenogram also revealed that the two permanent teeth (1+ and +1) were peg shaped



Fig The patient P B eight years old

Examination of the nose showed atrophic mucous membranes Both nasal cavities were wide with h avy crusting No special test of salivary or l chrymal gland function was made but the ecretion seemed to be normal

An experiment with careful heat provocation during continuous checking of the boy s temperature was performed By means of electric pads placed in the bed the boy was exposed to increased heat for one hour and fifteen minutes The rectal temperature increa ed during this time from 37 1 C to 38 5 C without any signs of perspiration except for very little on the front of the thighs Another child of the same age simultaneously exposed to the same heat provocation showed strong perspiration The rectal temperature of this child did not increase

Biopsy of the skin was performed from the palm of the right hand and from the ri ht axillar region for histopathologic examination In serial sections from the biopsy material of the palm no sweat gland tructures or sebaceous glands could be detected In biopsy material from th axilla no sweat glands were seen except one underdeve oped and no fully developed sebaceous glands could be d tected No pathological changes were seen in the epidermis and the underlying corium contained the normal amount of hair follicles (P A Hofer) (Fig 1)

Af er discharge from the hospital hyperpyrexia has been avoided through cooling measures

A follow up examination at the age of 8 years showed the same clinical picture as before (Fi 2) The dental and ke etal age were delayed by about three years but mental and physical development was normal Roentgenograms showed as earlier hypodontia in the upper jaw and anodontia in the lower jaw (Figs 3 and 4) A dental bridge had been

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In this paper two cases of ectodermal dysplasia are presented Both patients showed a very marked temperature instability

CASE REPORTS

Case P B a boy born September 22 1961 oldest of two children The parents and the younger child were healthy Wassermann's reaction of the mother was negative Pregnancy delivery and neonatal period were uncomplicated Birth weight was 3770 g and height 51 cm The boy's maternal grandmother and great grandmother had a number of congenitally missing teeth and the grandmother had also noticed diminished perspiration Otherwise they and their relatives were healthy

The boy's physical and psychomotor development was normal The parents noted early that he was very sensitive to heat exposure During warm and sunny days his temperature rose to 39 C without any signs of infection The mother noted that he could not perspire All the time he had severe nasal secretions and heavy crusting in the nose with a foul odour Furthermore at an age of [illegible] he had only two erupted peg shaped central primary incisors At this age he was admitted to the Department of Paediatrics at the County Hospital Lidköping

The physical examination revealed a normally developed boy without any infectious symptoms His weight and height were within the normal limits His ears were bowl shaped and protruding The eyelashes and eyebrows were scanty The scalp hair was fine blond and sparse His nasal bridge was flat and his lip protruding The finger and toenails were normal Beneath the eyes he had bilateral linear wrinkles His skin was dry and he had minor eczematoid lesions on the forearm

Haemoglobin concentration was 11 8 g/100 ml WBC was 9700 Differential leukocyte count showed a normal distribution MSR 11 mm PBI 7 0 μg/100 ml Cholesterol 171 mg 100 ml The serum protein electrophoresis was normal and so was the quantitative determination of immunoglobulins Cytogenetical

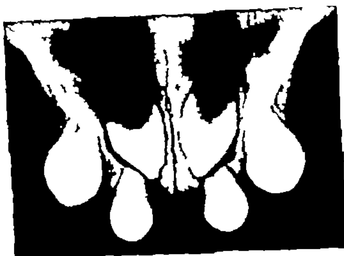


Fig 4 Roentgenogram of the upper jaw in case P B showing the peg shaped teeth

only few cilia were found The skull hair was fine blond and scanty (Fig 5) He had dry skin with slight eczematoid lesions on the cheeks and in the capillitium Small spots of pigment defects were noticed suprapubically The mucous membrane of the nose was atrophic Routine haematological investigations and serum protein electrophoresis were normal MSR was 11 mm Cytogenetical examination of peripheral leukocyte cultures revealed a normal chromosome constitution Roentgenographic examination of the upper and lower jaw showed no visible tooth buds

A biopsy specimen was taken from the right axillar region In serial sections no sweat glands could be detected Hair follicles and sebaceous glands were seen but some of the sebaceous glands were obviously small in size (P A Hofer) (Fig 6)

During the hospital stay his temperature several times rose to 38-39 C The boy's raised body temperature was easily controlled by sponging him with cooled water

In October 1968 when he was 10 months old increased production of tears was noted by the mother Tonometry revealed glaucoma on both sides and by gonioscopy bilateral malformation of the angle of the anterior chamber of the eye was diagnosed Because of this a bilateral goniotomy was performed Later a reoperation became necessary

An odontological investigation including roentgenographic examination of the parents and seven siblings was performed but neither hypodontia nor abnormal shape of the teeth have been recorded



Fig 5 Facial appearance of the child G Y at eight months of age

DISCUSSION

The two patients described presented all the main signs i e hypodontia hypotrichosis and hypo- or anhidrosis as well as a great number of minor signs of ectodermal dysplasia As minor signs many authors (1 12 16) have mentioned saddle shaped nose thickened and protruding lips malformed and protruding ears wrinkles around the eyes pigment disturbances and atrophic rhinitis These patients also often have eczematoid skin lesions similar to those described in the present patients Of the minor signs the patients presented here lacked only


a


b

Fig 3 Roentgenogram of the jaw in case P B Hypodontia in the upper jaw with peg shaped permanent teeth (1+ and +1) A dental bridge is inserted In the lower jaw no tooth buds are visible (*a*) Right side (*b*) Left side

made in the upper jaw when he was 7 years old A plastic operation on the protruding ears had also been performed

Case G J a boy born December 25 1967 The parents were from the Lebanon He had eight sib lings They were all healthy except one brother who was mentally retarded after an encephalitis Wasser mans reaction of the mother was negative The boy was born in Sweden one month prematurely His birth weight was 2780 g and height 47 cm There was no history of asphyxia

He was admitted to another hospital at the age of 2 months because of unexplained fever attacks and difficulties in feeding

The physical examination revealed protruding lips The skin was dry and with slight eczematous mani festations No infectious symptoms were detected Routine haematological and urinary investigations were normal MSR 11 mm Repeated bacterial blood cultures during attacks of high temperature were neg ative Examination showed the cerebrospinal fluid to be normal

During the 2 / months hospital stay the boy had a short attack of gastroenteritis and a local skin infection After treatment with antibiotics he became free of infection and it was then possible to observe him continuously during an infection free period During this period he had repeated attacks of fever up to 40 5 C when the temperature of the room was increased The child was discharged at the age of 5 months Two months later he was admitted to the Department of Paediatrics at the County Hospital in Lidkoping because of fever attacks without any other signs of infection

The physical examination revealed a small mouth protruding lips and a small nose No eyebrows and

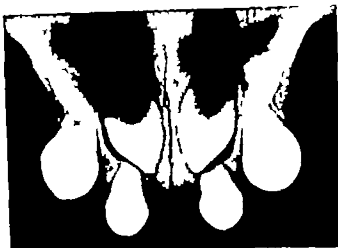


Fig. 4. Roentgenogram of the upper jaw in case P. B. showing the peg-shaped teeth.

only few cilias were found. The skull hair was fine, blond and scanty (Fig. 5). He had dry skin with slight eczematoid lesions on the cheeks and in the capillitium. Small spots of pigment defects were noticed suprapubically. The mucous membrane of the nose was atrophic. Routine haematological investigations and serum protein electrophoresis were normal. MSR was 11 mm. Cytogenetical examination of peripheral leukocyte cultures revealed a normal chromosome constitution. Roentgenographic examination of the upper and lower jaw showed no visible tooth buds.

A biopsy specimen was taken from the right axillar region. In serial sections no sweat glands could be detected. Hair follicles and sebaceous glands were seen, but some of the sebaceous glands were obviously small in size (P. A. Hofer) (Fig. 6).

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DISCUSSION

The two patients described presented all the main signs, i.e. hypodontia, hypotrichosis and hypo- or anhidrosis, as well as a great number of minor signs of ectodermal dysplasia. As minor signs many authors (1, 12, 16) have mentioned saddle shaped nose, thickened and protruding lips, malformed and protruding ears, wrinkles around the eyes, pigment disturbances and atrophic rhinitis. These patients also often have eczematoid skin lesions similar to those described in the present patients. Of the minor signs, the patients presented here lacked only



Fig. 5. Facial appearance of the child G. Y. at eight months of age.

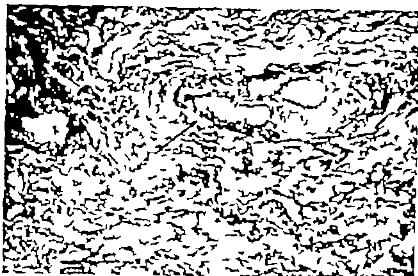


Fig 6 Section of skin (axillar region) from patient G Y showing no sweat gland structures but small sebaceous glands (→) (× 100)

prominent frontal bossing and in case G Y the nose was small rather than saddle shaped The nails as a rule are normally developed (1) and most often the patients are of normal intelligence (12)

Most striking was the instability of termo regulation Because they could not perspire both children developed high temperatures when the environmental temperature was increased In the skin biopsies performed no fully developed sweat gland structures could be detected microscopically A total absence of sweat glands or the presence of atrophic nonfunctioning glands is characteristic of ecto dermal dysplasia with anhidrosis In case P B it would probably be possible to detect sweat gland structures in the thigh region where per spiration was observed during the heat provo cation test In case G Y the very accentuated temperature instability speaks in favour of a complete anhidrosis The lack of ability to per spire can be very dangerous (9) Jespersen (12) even described a child who died from hyper pyrexia during his first year

Other gland structures can be involved for example the lachrymal and salivary glands The two cases described had no signs of im paired function of these organs The sebaceous glands can also be lacking but this is not a constant finding

Case G Y had a malformation of the angle of the anterior chamber of the eye on both sides resulting in bilateral congenital glau coma Malformations of eye structures of both ectodermal and mesodermal origin i e the lens the iris the cornea, the angle of the anter ior chamber of the eye are described in asso ciation with ectodermal dysplasia (4) How ever the malformation of the angle of the an terior chamber of the eye is rare and seems to be described in only a few previous cases of ectodermal dysplasia (4 6)

The peg shaped teeth seen in case P B are typical of the syndrome The dentition could be delayed or absent as in case G Y Because hypodontia can occur in other diseases these conditions must be considered in the differen tial diagnosis In incontinentia pigmenti (14) the shape of the teeth could resemble the peg shaped teeth of ectodermal dysplasia The skin changes however are of another type and the patients have no inability to sweat Ellis van Creveld s syndrome is a variant of anhidrotic ectodermal dysplasia Hypodontia may be pres ent but this syndrome is associated with poly dactyly chondrodysplasia and congenital heart disease (13) Hypodontia is also found in pa tients with hidrotic ectodermal dysplasia de scribed by Book (3) but these patients have hyperhidrosis and early whitening of the hair

The genetic transmission has in some inves tigations been described as a sex linked reces sive trait (5 12) Other studies (8 9) have shown a dominant transmission often with in

complete penetrans and variable gene expressivity De Silva stated (5) that ectodermal dysplasia is extremely rare among girls In the patients described here it has not been possible to prove fully developed ectodermal dysplasia among the parents or the siblings but in case P B both the maternal grandmother and the great grandmother had hypodontia and the grandmother also decreased ability to sweat The mother had peg-shaped upper lateral incisors which can be a manifestation variation of hypodontia However the frequency of peg shaped upper laterals in a Swedish population is as high as two per cent (7) In the family of P B inheritance of the trait may be sex linked recessive Thus the boy s mother may be a heterozygote conductor of the trait The mother and other female conductors have some stigmata but not a fully developed syndrome Another possibility is an autosomal dominant trait with variable expressivity

The treatment of infants with ectodermal dysplasia is symptomatic It is important to diagnose the condition as early as possible so that the parents can obtain information on how to prevent overheating Cooling measures during high environment temperature and fever attacks are advisable and can be life saving As in all cases of hypodontia it is necessary that dental development is checked by a pedodontist Fixed and removable prosthesis should be constructed for both functional and cosmetic purposes Regular contact with an otologist may be necessary in treating the atrophic rhinitis

SUMMARY

Two cases of ectodermal dysplasia diagnosed at the ages of 2 [illegible] years and 7 months are described The main signs were hypodontia hypotrichosis and hypo- or anhidrosis In both cases the temperature instability was a pronounced clinical problem In one of the two cases bilateral glaucoma was diagnosed at the age of 10 months

REFERENCES

1 Awwaad S & Essawy M E Hereditary anhidrotic ectodermal dysplasia *Arch Pediat* 77 496 1960
2 Blattner R J Hereditary ectodermal dysplasia Comments on current literature *J Pediat* 73 444 1968
3 Book J A Clinical and genetical studies of hypodontia *Amer J Hum Genet* 2 240 1950
4 Chosack A & Rosenzweig K Oligodontia associated with defects of the eyes *Oral Surg* 21 354 1966
5 De Silva P C C Hereditary ectodermal dysplasia of anhidrotic type *Quart J Med* 8 97 1939
6 Gasteiger H & Berthold H Ein Beitrag zur ektodermalen Dysplasie aus ophthalmologischer Sicht *Klin Mbl Augenheilk* 136 57 1960
7 Grahnén H Hypodontia in the permanent dentition *Odont Rev* vol 7 *Suppl 3* 1956
8 Harvold F Ectodermal dysplasi og anodonti *Norske Tannlægeforen Tid* 65 141 1955
9 Hippe E *Hereditary ectodermal dysplasia* of anhidrotic type with increased protein bound serum thyroxine *Acta Pædiat Scand* 56 687 1967
10 Holst G Dental treatment of patients with ectodermal dysplasia A report of five cases *Tandlægebladet* 67 60 1963
11 Ihre B Case of anodontia *Acta Med Scand* 79 [illegible] 1932
12 Jespersen H G Hereditary ectodermal dysplasia of anhidrotic type *Acta Pædiat Scand* 51 712 1962
13 Lodin H & Sjögren I Chondroectodermal dysplasia (Ellis van Creveld s syndrome) *Acta Pædiat Scand* 53 583 1964
14 Oldfelt V Incontinentia pigmenti *J Pediat* 54 446 1959
15 Rørvik R & Syrrist A Medfødt tannmangel forbundet med forandringar i hudsystemet. *Norske Tannlægeforen Tid* 49 237 1939
16 [illegible] & Montgomery H Hereditary anhidrotic ectodermal dysplasia *Arch Dermat Syph* 60 1170 1949

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

SCANDINAVIAN SOCIETY OF PAEDIATRIC PATHOLOGY

Meeting in Copenhagen Denmark June 12–14 1969

Aagot Christie Løken (Oslo Norway) *Poliodystrophia cerebri progressiva infantilis*

Poliodystrophia cerebri progressiva infantilis is one name for a cerebral degeneration characterized by a diffuse loss of neurons throughout the cortex with a corresponding reactive astrogliosis Involved to a lesser degree are the cerebellar cortex the thalamic nuclei the striatum and other nuclei A progressive atrophy involving the white matter as well follows in prolonged cases

The condition is seen in children who after a normal pregnancy and an uncomplicated delivery and postnatal period behave normally until a change in psychomotor development follows There is a regression often complicated with jerks rigidity and involuntary movements Convulsions often complicate the picture until the child dies decerebrated in opistotonus The condition lasts from weeks to months or years Two groups can be distinguished one infantile with symptoms starting before 1 year lasting up to 3 years of age the second or juvenile with an onset between 3 and 6 years lasting up to adult age

Case 1 A boy with a long history of vomiting and obstipation from 2–3 weeks of age gradually showed psychomotor retardation Convulsions were at no time observed He was hospitalized for malabsorbtion when he was 4 months old He was treated in a home for mentally retarded children and was readmitted to hospital under the diagnosis of gastroenteritis shortly before he died 2 years and 7 months old At *autopsy* there were no macroscopical changes in the viscera The brain weighed 1160 g appeared edematous with a moderately enlarged ventricular system and a thin corpus callosum Extensive neuronal loss was found all through the cortex with astroglial proliferation and small sudanophilic microglia Similar changes occurred in the striatum and globus pallidus In the cerebellum there was loss of Purkinje cells and fibrillary gliosis Some demyelination was seen in the temporal lobe

Case 2 A 17-month old boy with an unremarkable history until 16 months old when he had a cold with fever The same day he had tonic and clonic convulsions and was admitted in hospital where he became comatose EEG showed a diffuse mostly right sided dysrhythmia He became decerebrated and died after 4 weeks The histopathological changes were very similar to those reported in the first case except that the temporal lobes and hippocampus were spared

The etiology was discussed Anoxia is usually suspected although the first case gave no clinical indication for this Familial occurrence has been reported Of other possibilities were mentioned defective fetal development metabolic disorders infections virus lack of vitamin B_1

It was concluded that although anoxia could probably produce a similar picture there are several reasons for suspecting some other primary factor It is also possible that various metabolic or others pathogenetic mechanisms may result in similar morphological pictures

Arne Brun (Lund Sweden) *Aspects of cerebral development and maldevelopment of particular relevance to pediatric pathology*

Normal cerebral developmental features of premature and fullterm infants may simulate a pathological process These features are

1 Remnants of the periventricular matrix normally seen at fullterm consist of lymphocyte like neuroblasts and glial cells located lateral to the occipital and frontal ventricular horns and in the strio-thalamic groove Last to vanish toward the 4th postnatal month are perivascular collars of lymphocyte like cells in the frontal lobes The cerebellar nodulus often contains a mixture of nerve cells and undifferentiated cells

2 Around birth the subpial granular layer (SGL) of the cerebral molecular layer remains in the temporal lobes particularly the uncus sometimes in the frontal poles and the prepyriform areas where the cells may appear to invade the meninges It consists of glial and neuroblast like cells forming a layer subpially

3 Together with cellular and vascular meninges these features may simulate inflammatory changes tumour or retarded abnormal development

4 Densely cellular white matter is due either to a residue of the last migratory wave with cells arranged in waves or columns or the glial proliferation preceding myelin deposition Both features occur precisely where and when the common perinatal anoxic ischaemic lesions appear and thus may obscure or simulate such lesions

Against this background some maldevelopmental processes will be discussed

Migratory embryonal cells may be arrested and persist to form heterotopias These may be islands of relatively well differentiated gray matter or clusters of cells with varying degree of differentiation They form periventricularly (from matrix) in the white matter (from migratory waves) or the molecular layer (from the SGL) The paraventricular islands may be mistaken for parts of basal ganglia those closer to the cortex as deep parts of gyri Scattered heterotopic cells subcortically should be differentiated from normally occurring small numbers of subcortical nerve cells particularly occipitally SGL remnants may project into the meninges containing neurons The functional importance of heterotopias is unknown but they serve as indicator of arrested migration and concurrence of cortical malformation based on disturbed migration

The graver cortical malformations agyria and pachygyria are easy to discover and often combined with extensive heterotopias

The milder forms micropolygyria and mild dysplasia have structural abnormalities in common which are discussed under mild cortical dysplasia

Characteristic of micropolygyria are areas of abnormally small closely packed gyri without scarring and with normal consistency The gyri often are fused and may show 4 or 6 laminae with varying width Malformed areas often are symmetrically distributed

Mild cortical dysplasia the most common cortical malformation presents diagnostic difficulties Grossly the brain appears normal or shows some heterotopias and a suspicion of abnormally wide irregularly outlined cortex Microscopically the malformation is of varying following abnormalities

(*a*) abnormal cortical organization with poor to non existent lamination varying cell density abnormally placed or oriented neurons

(*b*) abnormal cortical maturation with poor differentiation of nerve cells few dendrites and retarded or abnormal cortical myelinisation Degenerative changes may add to the confusion of the cortical architecture

(Supported by Medical Research Council grant B68 12X 2037-02)

Edith Reske Nielsen & Helmer Søgaard (Aarhus Denmark) *Neuropathological changes in epidermolysis bullosa congenita dystrophica*

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fi ant according to the following histological features vesicular nuclei presence of nucleoli presence of cytoplasm presence of capsule necroses and calcification as well as the presence of mature ganglion cells

The importance of the first signs of histological differentiation in the tumor tissue was briefly discussed

The similarity of lesions reported in these 4 patients indicates that this i not a random combination of unusual conditions but a syndrome This is probably related to the more common often familially occurring combination of medullary thyroid carcinoma and bilateral pheochromocytomas

(Submitted for publication in Scandinavian Journal of Gastroenterology)

T Normann & B Otnes (O lo Norway) *Diffuse intestinal ganglioneuromatosis associated with tumors of endocrine organs*

A case of diffuse ganglioneuromatosis associated with mucosal neuromas medullary thyroid carcinoma and severe diarrhea is reported in a 15 year old boy To our knowledge only 4 cases with similar pathological changes in the intestine have been reported previously In 3 of these the intestinal lesion was associated with a medullary thyroid carcinoma mucosal neuromas bilateral pheochromocytomas and evere diarrhea Thick eyelids and lips gave these 3 and our own patient a peculiar facial similarity In one of the previously reported cases a positive family history was obtained In our patient the family history was negative No pheochromocytomas have yet been diagnosed in our patient but on one occasion slightly elevated values of urine catecholamine excretion was found

The diffuse ganglioneuromatosis was initially thought to be the cause of the diarrhoea However intestinal passage time did not decrease satisfactorily after colectomy After removal of his medullary thyroid carcinoma the intestinal passage time showed a marked increase and ileostomy loss a marked decrease Several biologically active substances uch as serotonin prostaglandin and calcitonin have recently been found in medullary thyroid tumours One of these might be responsible for the diarrhea seen in these patients Diarrhea is also a prominent symptom in some patients with medullary thyroid carcinoma without associated lesions

Edith Reske Nielsen H Søgaard & Aa Harmsen (Aarhus Denmark) *A case of generalized congenital toxoplasmosis*

Three weeks after a normal birth an infant developed somnolence and generalized rigidity The cranium was hydrochephalic and the fontanelles were tense The C S F contained 1800 mg protein but no cells There were large grayish opacities in the vitreous The X ray of the skull showed scattered intracranial calcifications Her temperature was periodically raised and she had repeated generalized tonic fits She died 40 days old

The normally developed brain weighed after fixation 515 g The leptomeninges were thickened and discolored and there were large yellowish depressed and softened cortical areas In the coronal sections there were necrotic and cystic regions and the walls of the ventricular system were thickened in some places necrotic in others The spinal cord was swollen Both eyes showed severe pathological changes with exudates retinal detachment sclerosis and thickening of the retina and the choreoidea and opacity of the vitreous

The l ptom ninges the brain and the spinal cord contained granulomatous areas often with a necrotic centre Between proliferating capillaries and fibroblasts were many phagocytes lymphocytes and a few plasma cells and eosinophilic leucocytes The macrophages contain numerous typical toxoplasma and many p eudocysts are pre ent In both the brain and the spinal cord there were places where the inflammatory reaction was diffuse and others where there were toxoplasma without reaction

Two cases (age 9 and 15 years) of epidermolysis bullosa congenita dystrophica are reported

The characteristic macroscopical and microscopical changes in the epidermis and corium were found but in addition with vascular lesions with intima thickening and calcification of all the layers In both cases a severe generalized amyloidosis had developed and the children died in uremia The peripheral nerves had signs of demyelinization and in one of the cases a severe clear-cut degeneration with loss of the myelin sheaths of the posterior columns was seen These changes have not been reported previously

A reasonable hypothesis is that the lesions of the skin and the nervous system are caused by a common ectodermal embryonal defect

H Søgaard & K Bertelsen (Aarhus Denmark) *Developmental tumors of the base of the skull in new born infants*

Four cases of benign congenital teratoma in newborn infants are reported In two cases the tumor arose from the base of the skull filled the entire buccal cavity and protruded from the mouth In another case the tumor was present occipitally and was associated with anencephaly and in the fourth case the tumor was *found in relationship with the tonsils*

The patients were operated on three are still alive (five months–two years after the operation) Incomplete development of the brain was seen in only one case and in contrast to other published cases of similar cases no congenital malformations were found in other parts of the body

The histological pattern of the tumors i verified by illustrations and the origin discussed

Judit Makinen (Helsinki Finland) Neuroblastoma *Histological classification of 54 cases*

Fifty four cases of neuroblastoma in children 0–14 years old were treated between the years 1950 and 1966 in the Children s Hospital University of Helsinki

Thirty two of these patients died within the first six months and nine within the second six months after the diagnosis was made and treatment was started The 13 children who survived at least one year were alive and without clinical evidence of disease in March 1969

The shortest follow up was three and a half years the longest 13 and a half years

The histological features of the biopsy and/or autopsy specimens taken from the primary tumor were retrospectively examined in all cases in an attempt to find significant differences between the fatal and non fatal cases

The following microscopical features were evaluated, either as indicating differentiation toward ganglioneuroma or as a better prognostic sign as reported by several authors

1 Increase in nuclear size with the presence of pale spherical often vesicular nuclei
2 Nucleoli
3 Slightly acidophilic cytoplasm
4 Cytoplasmic cell processes
5 Appearance of fibrillary intracellular material
6 Ball like clumping of the tumor cells
7 Presence of true rosette formations
8 Presence of fibrous capsule
9 Hemorrhages in the tumor tissue
10 Necroses in the tumor tissue
11 Calcification in the tumor tissue
12 Presence of ganglion cells

Histologically the tumors were classified into the following groups

Group I—no histological sign of differentation

Group II—various signs of histological differentation

The second group was divided into two parts

Group II *a*—without ganglion cells

Group II *b*—with the presence of completely differentiated ganglion cells

In the statistical analysis the difference between the fatal and non fatal cases was signi

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Judit Makinen (Helsinki Finland) Neuroblastoma *Histological classification of 54 cases*

Fifty four cases of neuroblastoma in children 0–14 years old were treated between the years 1950 and 1966 in the Children's Hospital University of Helsinki

Thirty two of these patients died within the first six months and nine within the second six months after the diagnosis was made and treatment was started The 13 children who survived at least one year were alive and without clinical evidence of disease in March 1969

The shortest follow up was three and a half years the longest 13 and a half years

The histological features of the biopsy and/or autopsy specimens taken from the primary tumor were retrospectively examined in all cases in an attempt to find significant differences between the fatal and non fatal cases

The following microscopical features were evaluated either as indicating differentiation toward *ganglioneuroma or as a better prognostic* sign as reported by several authors

1 Increase in nuclear size with the presence of pale spherical often vesicular nuclei
2 Nucleoli
3 Slightly acidophilic cytoplasm
4 Cytoplasmic cell processes
5 Appearance of fibrillary intracellular material
6 Ball like clumping of the *tumor cells*
7 *Presence of true rosette formations*
8 Presence of fibrous capsule
9 Hemorrhages in the tumor tissue
10 Necroses in the tumor tissue
11 Calcification in the tumor tissue
12 Presence of ganglion cells

Histologically the tumors were classified into the following groups

Group I—no histological sign of differentiation

Group II—various signs of histological differentation

The second group was divided into two parts

Group II *a*—without ganglion cells

Group II *b*—with the presence of completely differentiated ganglion cells

In the statistical analysis the difference between the fatal and non fatal case was signi-

B Robertson & B I Ivemark (Stockholm Sweden) *Abnormalities of the costochondral junction in cases of perinatal death with special reference to hyaline membrane disease*

Microscopic examination of decalcified sections from the costochondral junction in a consecutive series of 70 neonatal autopsy subjects revealed changes in 76 per cent In 32 per cent the lesion was non specific and termed chondral dysplasia most of these instances being only slightly affected In 22 per cent the metaphysis had a porous appearance displaying osteoclasts reduced hematopoietic activity and hypovascularity giving the area the appearance of trabecular rarefaction A third group (22 per cent) had an intermediate type of change In altogether 58 per cent of the abnormal specimens the abnormal metaphyseal zone was demarcated clearly visible in low power fields and measuring 500–1500 microns The size of this reactive zone was not correlated to the degree of prematurity or to the time of postnatal survival However the presence of this zone showed a marked correlation to the occurrence of pulmonary hyaline membranes suggesting a common etiologic factor operating several days before birth

References

1 Emery J L & Kalpaktsoglou P K *Arch Dis Childh* 42 1 1967
Robertson B & Ivemark B I *Acta Path Microbiol Scand* In press

H Søgaard K W Kastrup & Edith Reske Nielsen (Aarhus Denmark) *Hydranencephaly*

The clinical features in a girl with hydranencephaly is reported The pregnancy was uncomplicated The child was born as the second in a pair of twins The twin brother is healthy Respiratory distress developed in the first 24 hours The child failed to thrive and developed signs of internal hydrocephalus death occurred at the age of 4 months Neurological examination demonstrated persistence of neonatal automatisms including grasp Moro and stepping reflexes There was loss of vision EEG showed absence of electrical activity over the major part of the brain Echoencephalograms failed to demonstrate ventricular echoes In the pneumoencephalograms there was a free floating airbubble Transillumination of the head caused it to glow red all over and was found to be a simple test in confirming the diagnosis as did a decreased skin temperature on the skull Poikilothermia was a prominent feature

Spontaneous motoric function decreased and as the condition deteriorated an increased flexortonus was observed with twitching spasms Intrauterine infection with toxoplasma and cytomegalic virus was ruled out

The loss of distal phalanges on 2 fingers and a systolic murmur indicating a septal defect was also observed Isostenuria and inability to increase the osmolality of the urine in a salt loading test (Cottet) was observed Injection of antidiuretic hormone corrected this and thereby confirmed the diagnosis of diabetes insipidus

The glucose tolerance test was normal and also the insulin provocative test Determination of secretion of growth hormone as stimulated by hypoglycemia revealed a normal response suggesting the persistence of at least some function in the anterior pituitary lobe

Angiography showed a closure of the internal carotid vessels just below the entrance into the skull and persistence of a functioning vertebral basilar system supporting the theory of a possible insufficiency as an etiological factor

Autopsy

Four month old girl weight 4350 gr length 55 cm The second and third left finger had no distal phalanges but other external congenital malformation was not observed The cranium with moderate hydrocephalus measured 47 cm A congenital heart malformation was found consisting of valvular aortic stenosis septum primum defect hypertrophy of the right ventricle and a persistent ductus arteriosus but no other congenital malformations were observed in the organs The adrenal glands were normal

The cranial cavity contained 300 cc xantho-

Many areas were encrusted with calcium salts There were severe degenerative changes of the ganglion cells and nerve fibres throughout the central nervous system Around miliary granulomas there were swollen astrocytes and perivascular infiltrations of lymphocytes Both eyes had severe chorioretinitis with destruction and necrosis of the retina and many toxoplasma in cysts

Dorthe Francis & J C Melchior (Copenhagen Denmark) *Toxoplasmosis acquisita a case report*

Toxoplasmosis acquisita is usually a benign infection Only a few fatal cases have been reported Sabin (1) Pinkerton & Henderson (2) and Sexton *et al* (3) and the patients in question died from acute primary infection

The following case is reported because of an unusually long history of acquired toxoplasmosis terminating in death after a minor operation

Case report

An eight year old boy was admitted to hospital with abdominal distension Four years previously he had been hospitalised for acquired toxoplasmosis diagnosed on the basis of a high toxoplasmosis neutralisation test (1 1250) a positive culture from lymphnodes and histological changes in the same lymphnodes similar to those caused by toxoplasmosis Subsequently he had enlargement of lymphnodes, abdominal distension with hepatomegaly periods of fever enteritis and atypical pneumonia chronic sinuitis and middle ear infection Adenectomy was carried out without immediate complications After a few days of raised temperature however he suddenly developed hyperpyrexic shock and died within a few hours

Laboratory examinations were within normal limits except for the very low gamma globulin fraction of the plasmaproteins

No pathogenic bacteria were isolated from blood tissue (lymphnodes) urine or feces cultures for M tuberculosis were also negative

The autopsy findings were a firm atelectasis in the right lung hepatomegaly and multiple ulcerations in the intestinal tract with fibrin on the peritoneal surface and fibrous adhesions The pancreas was fibrotic with retroperitoneal fibrosis occluding the inferior vena cava and right ureter There was generalized lymph node enlargement The thymus could not be detected in the mediastinum The central nervous system was macroscopically normal

Microscopically there were chronic interstitial pneumonia and slight lymphocyte infiltration of the myocardium and the liver In the intestinal tract and the lymphnodes there were changes in the lymphatic tissue with fibrosis a marked sinus and follicular reticulosis, small necroses and numerous large macrophages with PAS positive granules in the cytoplasm In the pancreas and the retroperitoneal space there were fibrosis and pronounced infiltration with lymphocytes and plasma cells Most of the glandular tissue in the pancreas had disappeared leaving irregular insulae and a few normal ducts In the mediastinum small islands of hypoplastic thymic tissue were seen In the brain there were slight and diffuse degeneration of ganglion cells gliosis and lymphatic infiltration A single small necrosis was observed incorporating a circular structure about 20 μ in diameter and containing numerous granules somewhat similar to toxoplasma bodies

At autopsy no pathogenic bacteria including M tuberculosis were isolated from blood tissue or feces and toxoplasma organisms were cultivated only from the brain

The unusual course of acquired toxoplasmosis in this boy and his sudden death after a minor operation may be explained as a result of an immunological deficiency state causing the severe widespread chronic inflammation

References

1 Sabin A B *JAMA 116* 801 1941
2 Pinkerton H & Henderson R G *JAMA 116* 807 1941
3 Sexton R C Eyles D E & Dellman R E *Am J Med 14* 366 1953

it is suggested in early phase of sulfatidosis to treat the patient with a vitamin A deficient diet (1)

The lack of β D galactosidase was found in the kin of three Greenland children (Table 1) clinically characterized by a slightly increased excretion of AMP bone defects—especially in cristae iliaca as verified by X ray examination —and by the clinical finding of co-sanguinity of the parents (2 5) The lack of β D galactosidase in these case supplements van Hoof & Hers (10) list of mucopolysacchari doses with known ly osomal defects (fucosi dosis generalized gala tosidosis mannosidosis elective lack of galactosidase in everal but not all organ and the elevated gala tosidase disorder) The c data also agree with those of Öckerman & Kohlin (11)

References

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2 Clausen J Dyggve H V & Melchior J C *Arch Dis Childh* 38 364 1963
3 Clausen J Melchior J C & Dyggve H V *Arch Dis Childh* (to be published)
4 DeDuve G The Lysosome Concept *Ciba Symp on L sosomes* Churchill Ltd London 1963 pp 1 31
5 Dyggve H V M lchior J C & Clau en J *Arch Dis Childh* 37 525 1962
6 Lou Christensen H O & Clausen J *Clin Chem Acta* 18 465 1967
7 Melchior J C & Clausen J *Acta Ped at Scand* 57 2 1967
8 Novikoff A B Lysosomes in the physiology and pathology of cells *Ciba Symp on Lysosomes* Churchill Ltd London 1963 p 36
9 van Hoof F & Hers H G *Rev Internat He patol* 8 815 1967
10 — *European J Biochem* 7 34 1968
11 Öckerman P A & Kohlin P *Acta Pediat Scand* 57 281 1968

Torben Schmidt & K Mauritzen (Copen hagen Denmark) *Hepatoblastoma and hepa tocarcinoma in infancy and childhood*

The need for a reliable histological classifica tion of malignant tumors of the liver in infancy has increa ed during the last years since the resul s of surgical treatment of hepatoblasto mas are by far much better than tho e of he patocarcinomas

A series of 6 cases of hepatoblastomas and one ca e of h patocarcinoma in children ad mitted to the Rigshospitalet of Copenhagen during the last 10 years is presented

Hepatoblastomas

Age and sex 5 of the cases were in boys and one in a girl all ranging in age from two months to two and a half years

Si e localization and gross anatomy The average size was about 10 cm in diameter ex cept in the case of the boy of two months whose tumor measured 3 cm The tumors were localized to the right liver lobe except in one case They appeared gro sly lobular and more or less encapsulated

Classification After Willis the tumors were divided in *pure epithelial* and *mixed* i e tu mors with an epithelial as well as a mesen chymal component

Microscopic appearance In both epithelial and mixed tumors two epithelial types were encountered

(*a*) *Embryonal type* with very little cellular differentiation corresponding to the very early phase of liver embryogenesis The cells were small dark with a high mitotic rate and often arranged in rosette formation Extramedullary hemopoiesis was not found in the areas of this cellular type

(*b*) *Fetal type* had the appearance of the pre natal liver tissue arranged in liver plates The cells were *rather light* with a lesser nucleus cytoplasm ratio than the former type Extramedullary hemopoiesis was often en countered

The *mesenchymal component* of the mixed type consisted ordinarily of loose and primi tive connective tissue or of osteoid like tissue wh reas cartilage was not found

Hepatocarcinoma

This tumor was diagnosed in only one case in a 14 year old girl The histological picture was

Table I *Lysosomal enzymic activities of dermal biopsies*[1]
Activity × 10⁻³ measured in μmoles/min/mg protein

Dermal biopsies Lysosomal enzymes	M J H J	H S O M J	L A A A J	Normal mean
Substrates				
1 p Nitrophenyl α D manno pyranosidase	2 69	6 34	3 97	0 84 ± 0 33
2 p Nitrophenyl N acetyl β D glucosaminidase	25 7	30 61	17 29	28 9 ± 2 5
3 p Nitrophenyl β D galacto-pyranosidase	0	0	0	7 1 ± 1 0
4 p Nitrophenyl α l fuco pyranosidase	2 66	8 45	6 92	15 7 ± 3 1
5 p Nitro phosphatase	75 33	124 95	70 3	156 ± 11
6 p Nitrophenyl β D glucuronidase	1 77	4 22	4 61	5 6 ± 0 4

[1] Cf Clausen J Melchior J C & Dyggve H V (1969)

chrome cerebro spinal fluid a normally developed falx cerebri and tentorium cerebelli with *scattered hemorrhages Only the basal parts* of the temporal lobes the hippocampus and the eyes were present of the prosencephalon The cerebellum the brain stem and the cranial nerves were normal It was possible to identify most of the cerebral arteries in the thin layer of cerebral tissue at *the base of the middle* cerebral fossa In the sella turcica a small hypophysis was found but only the adenohypophysis was intact the neurohypophysis was totally replaced by collagenous tissue The pathogenesis of this peculiar malformation was discussed and the case documented by *illustrations*

Jørgen Clausen Johannes C Melchior & Holger V Dyggve (Copenhagen Denmark)
Progressive encephalopathies

The original classification of progressive encephalopathies associated with storage of polar lipids and/or polysaccharides has been based on the symptomatology and the type of chemical material stored In using this classification *certain clinical* entities e g the mucopolysaccharidoses showed overlapping chemical abnormalities Excretion of acid mucopolysaccharides (AMP) in these diseases involved excretion of chondroitin sulfate B alone or associated with excretion of keratosulfate and/or heparitin sulfate Furthermore these entities similar to the infantile form of gangliosidosis were often seen in connection with storage of mono sialoganglioside in the central nervous system

After the discovery of the lysosomes (4 8) and after the demonstration that the so-called thesaurocytes found in Hurlers syndrome are identical with swollen lysosomes (9) it has been possible to classify lipidoses and muco *polysaccharidoses as* lysosomal disorders with lack of a lysosomal enzyme degrading the stored material

The compounds with the highest colloid content e g tri sialogangliosides (possibly protein bound) and AMP protein bound by a car*bohydrate bridge* of one or more monosaccharides (fucose mannose arabinose) are degraded by a stepwise liberation of sialic acid saccharides and protein The degradation is catalyzed by lysosomal enzymes Corresponding to most of the enzymic steps disorders have been characterized by lack of the corresponding enzyme

Of the disorders of lysosomal pathogenesis sulfatidosis (lack of sulfatide sulfatase) and lack of β-D galactosidase are demonstrated in the present communication (cf 3 7) Sulfatidosis was biochemically traced in family studies by quantitative thin layer chromatography (6) of lipids extractable from urinary sediment and serum and by enzymic determination of lysosomal enzymes These methods revealed genetic carriers of the disease *Since vitamin A* is necessary for the formation of active *sulfate* (PAPS)—one of the precursors *of sulfatide*—

it is suggested in early phase of sulfatidosis to treat the patient with a vitamin A deficient diet (1).

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References

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6. Lou Christensen H O & Clausen J. *Clin Chem Acta* 18 465 1967
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A series of 6 cases of hepatoblastomas and one case of hepatocarcinoma in children admitted to the Rigshospitalet of Copenhagen during the last 10 years is presented.

Hepatoblastomas

Age and sex. 5 of the cases were in boys and one in a girl, all ranging in age from two months to two and a half years.

Size, localization and gross anatomy. The average size was about 10 cm in diameter except in the case of the boy of two months whose tumor measured 3 cm. The tumors were localized to the right liver lobe except in one case. They appeared grossly lobular and more or less encapsulated.

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5. Dyggve H V. Melchior J C & Clausen J. *Arch Dis Childh* 37 525 1962
6. Lou Christensen H O & Clausen J. *Clin Chim Acta* 18 465 1967
7. Melchior J C & Clausen J. *Acta Pediat Scand* 57 2 1967
8. Novikoff A B. Lysosomes in the physiology and pathology of cells. *Ciba Symp on Lysosomes* Churchill Ltd London 1963 p 36
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Age and sex. 5 of the cases were in boys and one in a girl all ranging in age from two months to two and a half years.

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Hepatocarcinoma

This tumor was diagnosed in only one case in a 14 year old girl. The histological picture was

that of adult hepatoma with a well pronounced polymorphism and with many giant tumor cells

Follow up

Two cases of hepatoblastoma survived a surgical resection with a follow up of 24 months and 29 months respectively Two died of cardiac arrest during operation one died of the tumor two months after operation and one case was not followed but the patient was in poor condition when discharged from hospital

The girl with hepatocarcinoma died of cardiac arrest during operation There were metastases to the retroperitoneal lymph nodes

One case of hepatoblastoma showed an *endocrine disorder* On admission a boy one and a half years old with hepatomegaly had precocious puberty with pubic hair development and elevation of urinary gonadotrophins to 9 i u After surgical resection of the tumour microscopically a mixed hepatoblastoma the activity had fallen to less than three units Half a year after the operation this normal value was maintained and the pubic hairs had disappeared The boy was completely healthy one year after the operation

Jørn Engel Møller (Copenhagen Denmark)
Diagnostic value of cortex biopsy

There has been increasing interest in cerebral cortical biopsy studies as the results obtained through this procedure are valuable for diagnosis prognosis and research in neurology neurosurgery psychiatry and in related basic science

As the post operative risks of infection hemorrhage and epilepsy are negligible and as the technical laboratory possibilities are increasing the clinicians are more frequent interested in cortical biopsy examination as an adjunctive diagnostic procedure

The term cortical biopsy is used even if it means a biopsy which includes an adequate amount of leptomeninges, cortical grey matter and subcortical white matter

The literature on the subject is scarce

As Biemond (1965) points out after collecting the opinions of experienced neurologists in Europe and U S A the following criteria must be fulfilled regarding the types of disease in which cerebral biopsy may be concidered

1 The manifestations of the illness must clearly point at a diffuse cerebral disorder accompanied by dementia
2 The course of the disease must be unmistakably progressive
3 The clinical diagnosis must have remained uncertain despite of employment of all indicated neuroroentgenological and clinical methods including serological chemical and microscopical examination of fluids and in special cases including biopsies from muscles peripheral nerves and rectum

In the future the above criteria may have to be extended because evolution in biopsy studies outside the brain has shown that the indication are widened especially with regard to research

It must always be remembered that the procedure involves the most precious of the human resources For this reason it is assumed that the entire problem is discussed by the clinical staff the surgeon the pathologist the *chemist and the ultramicroscopist*

The biopsy has to be done with the patient in general anesthesia It is very important that a block of brain tissue measuring 1 cm³ to 1 5 cm³ in size including the leptomeninges cortex and subcortical white matter is non traumatically sharply excised

Ventriculography can be carried out coincidently with the brain biopsy

An optimal biopsy specimen is divided for the following examinations Histological histochemical electronmicroscopical and biochemical study

The present material consists of 180 cortical biopsies Here the pre biopsy clinical diagnosis has been compared with the neuropathological diagnosis

The biopsy was considered to be of value if

it either confirmed or made a new diagnosis. *138* of the *180* biopsies had histological abnormalities—40 showed inflammatory changes, 11 degenerative, 13 presenile, senile and arteriosclerotic changes. In 11 cases developmental abnormalities were present. Two had anoxic, 50 non-specific degeneration and in 10 biopsies no definite diagnosis could be given due to artefacts. 42 biopsies were normal and in one case leptomeningeal and cerebral carcinomatosis was present.

In the present material 78 of 180 were of value since they fulfilled the abovementioned criteria.

B. I. Ivemark

Acta Pædiat Scand 59 110–117 1970

PROCEEDINGS OF PAEDIATRIC SOCIETIES

THE FINNISH PAEDIATRIC SOCIETY

Meeting May 27 1969

Tadeusz Chrapowicki (Warsaw Poland) *Polish mother and child welfare*

The population of Poland is c 33 5 million One half lives in rural areas and $^{1}/_{3}$ is children

There were 522 000 live births during last year The pediatric welfare work takes care of children up to 18 years

The total number of physicians exceeds 40 000 that of pediatricians 5000 and that of gynecologists and obstetricians 3000 There are 14 pediatricians per 100 000 habitants

There is a continuously increasing number of pediatricians working in narrow subspecialities pediatric surgery cardiology contagious disease Tb laryngology endocrinology psychiatry nephrology oncology etc

The total number of children s hospital beds is 32 806 that of newborn s beds in special wards 13 500 The number of gynecologic and obstetric beds is 23 000

Actually 77 per cent of the deliveries take place in hospitals 18 per cent in rural labour rooms and 5 per cent at home under the ca of a specialist doctor and midwife

Family planning is encouraged by the go ernement Prophylactic examinations of women for cancer have been made in / all provinces

Paralelly to the development of the hospi care the ambulatory care extends its activi both in general practice and in different sp cialities In the central towns of the medi districts more than 20 different specialities a covered

We pay special attention to the developme of sanatoria for the treatment of chronic di eases in children The number of beds in san toria is 8000 Children s mortality has d creased to 33 4 per 1000 live births

The number of patients with tuberculosis permanently diminishing in 1969 20 ne cases were registered per 100 000 children be low 14 years of age

Two thirds of the schools have own phys cians and 1 200 000 school children were se to summer camps

Meeting May 30–31 1969

(together with the Polish Paediatric Association)

SYMPOSIUM OF THE NEWBORN

Irena Żywicka Twarowska (Poznan Poland) *Electroencephalogram of the asphyctic newborn*

A group of 122 newborn infants asphyctic at birth and expressing symptoms indicating brain damage were studied together with a contro group of 127 healthy newborns The results o EEG investigations recorded 2 to 6 times in every newborn were correlated to etiologi factor of neonatal asphyxia clinical and neu rological picture of the newborn additional ex

minations gross anatomical and histopathological findings at necropsy and follow up of the infant s development

Ninety six of 122 newborn babies survived and about half of them showed disturbances in their psychomotor activity The author demonstrates various types of pathological EEG tracings during the first days of life (paroxysmal features slow activity poor activity marked focal abnormalities abnormal sleep pattern etc) and discusses their causes and electrogenesis EEG investigations during the neonatal period are of importance with respect to (1 early diagnosis of cerebral damage 2) prognosis concerning the newborn s life and sometimes later development Their prognostic value is dependent on persisting abnormal tracings The best diagnostic and prognostic information is provided by EEG recording started during the first two days of life

Discussion

N Hallman Do you always find a normal EEG in normal babies?

I Twarowska The EEG of the newborn is showing great variations depending on the state of activity light sleep or deep sleep but paroxysmal features never occur in healthy newborns

Zofia Szczepanska (Poznan Poland) *Acid base balance during exchange transfusion in newborn infants*

Acid base balance was assessed in 18 newborn infants to whom 24 exchange transfusions were performed because of hemolytic disease and hyperbilirubinemia of prematurity The series was divided into two groups according to clinical condition The first group consisted of 9 newborns with moderate signs of hemolytic diseases but good general condition In the second group there were 9 infants with severe hemolytic disease and poor general condition at birth and at the beginning of the transfusion Fifty seven vials of citrate blood used for transfusion were also checked the mean pH being 6.50

In the first group disturbances in acid base balance were found in 3 cases prior to in 6 cases during and in 6 cases after the transfusion whereas in the second group disturbed acid base balance was found in nearly all cases Respiratory and metabolic acidosis was found in infants of the second group prior to the transfusion Variations of acid base balance during transfusion and a distinct tendency towards alkalosis after tranfusion were found in almost all newborns

D Lomnicka I Herbst J Gurda & M Szostakowska (Warsaw Poland) *Estimation of various chemical and serological factors in the amniotic fluid its relationship to hemolytic disease of the newborn*

Various examinations of the amniotic fluid were compared to evaluate their suitability for assessing the degree of foetal affection due to blood group incompatibility

Fourty nine specimens of amniotic fluid from 36 Rh immunized cases were examined The series was divided into 3 groups according to optical density of the amniotic fluid

The general condition of the newborns corresponded well to the optical density of the amniotic fluid The mean bilirubin concentrations of the two groups were significantly different A correlation between bilirubin concentration and optical density could not be confirmed

Anti D antibodies occurred in some cases in all groups irrespective of the level of antibodies in the mothers blood

The acid base balance of the amniotic fluid was normal in all groups

It was concluded that the optical density is the only one of all our examinations of the amniotic fluid which provides useful evaluation of the affection of the foetus in a pregnancy complicated with Rh immunization

T O A Pmvilainen (Turku Finland) *Foetal tracheobronchial fluid*

The chemical composition of the tracheo bronchial fluid is different from that of the amniotic fluid In the foetal lamb it resambles chemically more the plasma than the amniotic fluid

In our studies sheep and guinea pigs were used as experimental animals The fetuses were delivered by Caesarean section sheep under local and guinea pigs under barbiturate anesthesia The lowest glucose concentration in both species was observed in the tracheobron chial fluid In the amniotic fluid of the sheep the glucose concentration was more than four to five times as high as in blood samples When the glucose level of the maternal blood in the guinea pigs was changed by intravenous glu cose infusion insulin and glucagon the effect on the glucose concentration of the tracheo bronchial fluid of the foetus was very slight during an observation time of one to one and a half hours Significant differences in choles terol and creatinine concentrations in the foetal and maternal blood amniotic fluid and tracheobronchial fluid were found in both spe cies (Pmvilainen *et al* 1967)

Varying directions of the concentration dif ferences of several substances indicate that the formation of the tracheobonchial fluid cannot be explained as a result of one factor only Too little is known of the foetal pulmonary trans-capillary passage to explain the possible trans-port of water and other substances between blood and peripheral airways

Reference

Paavilainen T O A Hirvonen L & Peltonen T Glucose cholesterol and creatinine in the fetal tracheobronchial fluid of the guinea pig and the sheep *Acta Paediat Scand* 56 660 1967

Krystyna Buchcar Halon (Warsaw Poland) *The role of separate neonatal hospital units in the decrease of mortality*

The Warsaw medical district accounts for nearly 1/11 of the Polish territory with a population of c 2 5 million the mean number of live births per year was 41 860 in 1964–1968

In 1960–1964 infant mortality varied from mortality there was a particularly serious situation at that time i e a considerable number of cases of perinatal trauma and of various diseases

In 1964 an obstetrical gynaecological d partment was opened at the Warsaw medical district central hospital with separate beds for cases of abnormal pregnancy There were 50 beds for newborn and low weight infants and 30 unspecialized district hospitals The activi ties of the above mentioned departments in th field of prophylaxis and treatment intense care and training of the hospital staff resulted in a significant decrease in infant mortality from 54 8 to 38 0 The perinatal mortality decreased from 38 0 to 28 5 and the postnatal mortality from 20 4 to 15 7

Eero Vapaavuori (Helsinki Finland) *Respi rator treatment in respiratory failure of the newborn* (To be published in *Acta Paediat Scand*)

Tom Sederholm (Helsinki Finland) *Late prognosis of foetal erythroblastosis*

Although the cytotoxic effect of bilirubin is confirmed it has never been proved that bili rubin is the only cause of kernicterus The pos sible occurrence of less severe cerebral injury following erythroblastosis has not been exten sively investigated

A retrospective study of children treated be cause of foetal erythroblastosis at the Chil dren s Hospital University of Helsinki in 1963–1964 was started Of the 301 children who survived the neonatal period 92 per cent could be traced by the time of this preliminary report One child was excluded because of Down s syndrome

The parents of all children were requested to fill in a questionnaire about the development of their child and the possible occurrence of motor hearing speech psychological and other disturbances

The mean age at learning to walk was 12 7 months which is about 1 month later than the average for Finnish children Twenty seven per cent learned to walk at the age of 14 months or later The mean age at learning to talk sentences was 19 9 months and 25 per cent spoke their first clear sentences at the age of 21 months or later At the age of 4–5 years one child suffered from athetosis and three from spastic hemiparesis In addition 17 per cent were thought to be clumsy Speech disturbances occurred in 40 per cent 28 per cent tired easily when playing and 11 per cent had a short attention span Eight per cent of the children were described as hyperactive Lability breath holding spells and antisocial behaviour occurred in 4 per cent Four per cent of the children had enuresis and 0 4 per cent had encopresis

Fifty four children with a low cord hemoglobin level were compared to 53 children with a high value and a clear difference in the development was found even when these main groups were divided into sub-groups on the basis of the maximal bilirubin values recorded during the neonatal period In the group with cord hemoglobin below 10 g per 100 ml and maximum bilirubin over 20 mg per 100 ml the mean age at learning to walk was 14 0 months while the group with low hemoglobin and bilirubin below 20 mg per 100 ml walked at the mean age of 13 1 months Children with hemoglobin above 15 g per 100 ml and maximal bilirubin over 20 mg per 100 ml walked at the mean age of 11 9 months while those with bilirubin below 20 mg per 100 ml walked at the mean age of 12 4 months The mean ages at the time of the first clear sentences in these groups were 23 0 21 2 20 3 and 19 5 months respectively

The results seem to indicate that minimal brain dysfunction frequently develops after foetal erythroblastosis and this dysfunction is related both to cord hemoglobin and to the maximal bilirubin level

In a closer follow up study the children with cord hemoglobin below 10 g per 100 ml were compared with those with hemoglobin over 15 g per 100 ml The neurological part of this investigation included a thorough neurological examination motor development tests according to Oszeretsky and EEG The psychological examination included intelligence tests as well as perceptual function and visual motor co-ordination tests A Rorschach test was done and phoniatric otoneurological and opthalmological examinations were also performed

So far 47 children have been examined The preliminary results clearly indicate that although the classical kernicterus syndrome is prevented by the treatment based on the criteria for exchange transfusions presently generally accepted this treatment is not sufficient to prevent the occurrence of slight disturbances such as tremor ataxia hyperreflexia over all clumsiness and co-ordination disturbances Crossed laterality of the dominant hand and foot occurred as did disturbances in perceptual functions and visual motor co-ordination The EEG showed frequently slight changes in the background activity but also in some cases hyper synchronous discharges Slight disturbances in vestibular function and dysfunction of eye movements were common in these children Disturbances of phonation such as hyperkinesia dysarthria and dyslalia occurred in a very high percentage of the children

It was observed that the lower the cord hemoglobin was the more frequent were the manifestations of minimal brain dysfunction regardless of the maximal bilirubin value recorded in the neonatal period

Clear evidence was presented that the observed abnormalities might be prevented by immediate exchange transfusions performed within the first hours of life

Irena Żywicka Twarowska K Jezykowska Zofia Szczepinska & A Bręborowicz (Poznan Poland) *Late prognosis in the pathologic condition of the newborn*

The purpose of this study was the evaluation of factors permanently damaging the newborn s

organism The subject of investigation were 552 children under observation from the neonatal period up to 4–7 years of age With respect to the young age this is only a preliminary report

I In the group of 75 infants with severe haemolytic disease abnormal development was found in 13 3 per cent The observations *showed that the association of even severe* hyperbilirubinaemia reaching the level of 25–30 mg per 100 ml with permanent damage of the child was not as apparent as expected In addition to hyperbilirubinaemia other factors such as anoxia intracranial haemorrhage hypoglycaemia immaturity dystrophy infections and disturbed adaptation may cause a permanent damage of the central nervous system (CNS)

II In the group of 115 infants delivered by toxaemic mothers abnormal development was found in 9 6 per cent Perm anent lesions in this group of infants could not be proved to result from toxaemia of pregnancy probably because the mothers were treated long time before delivery There was a probable correlation between disturbed development of the child and intrauterine dystrophy of the foetus In the neonatal period there was an evident relation between chronic toxaemia of pregnancy and intrauterine dystrophy of the newborn Thus intrauterine dystrophy was observed in 60 5 per cent of pregnancies complicated by chronic and in 13 5 per cent of those with acute toxaemia Additional analysis of small for date newborn babies revealed that toxaemia of pregnancy was the cause of dystrophy only in 28 6 per cent

III In the group of 122 infants born in a severe state of asphyxia with Apgar score 0 to 2 points abnormal development was found in 21 3 per cent The following obstetric factors bear a high risk for the baby foetal anoxia in cases of placenta praevia or prolapsed cord infections and abnormal foetal position and labour mechanism In this group the worst prognosis concerning the later development is occuring with postnatal apnoea lasting for more than 5 minutes or the presence of severe neurological symptoms persisting over 7 days of life

IV In the group of 140 infants with severe damage of the CNS in the neonatal period late abnormal development was found in 61 4 per cent Specific clinical symptoms persisting for more than 7 days of life appear to be of bad prognostic significance In most cases the first breath was not delayed In 10 per cent of the cases coexistance of a congenital malformation of the CNS was found

In the control group of 100 healthy newborn abnormal development was found in [illegible] infants

Ossi Pettay (Helsinki Finland) *Newborn infections*

At the Children s Hospital University of Helsinki several hundred exchange transfusions are done annually These transfusions are performed through an umbilical venous catheter Since umbilical catheterization is considered to include a risk for infection all these patients recieve antibiotics profylactically Bacterial swabs were taken from the umbilicus and bacterial culture of the blood drawn through the catheter at the beginning and after the exchange transfusion to evaluate the justification of this profylaxis The results of the swabs and cultures correlate quite well If the samples are taken during the first day after birth less than 10 per cent of the umbilical swabs and blood cultures are positive The percentage increases to approx 60 on the fourth day of life Blood cultures taken from a periferal vein on the third day of life revealed bacterial growth in 10 per cent In most but not all cases the bacteria were the same as found in the umbilicus

Generally speaking staphylococcus aureus has disappeared as a troublemaker in our newborn wards The gram negative bacteria Pseudomonas Klebsiella and Coli have taken their place Some of the strains possess a transferable resistance against at least ampicillin and

tetracyclin The clinical importance of this finding is so far very hard to evaluate The typing of the Pseudomonas strains has not revealed any special pathogenic strains In contrary one gets the impression that even strains belonging to normal bacterial flora are able to cause disease in these small infants

Irena Żywicka-Twarowska & K. Wiodarczak (Poznan Poland) *Newborn infections*

The results of systematic bacteriological studies performed during a period of 9 months on newborn infants and their environment at the Department of Newborn Pathology were presented

Gram negative bacteria were prevalent in the newborns and like Staphylococcus aureus they beared an extremely high risk for the infant The incidence of E coli was of an endemic character and various strains were found During a period of rapid rise in incidence of these bacteria resembling an epidemic haemotoxin producing strains were predominant and a carrier was found among the staff At the beginning of the study the incidence of Pseudomonas aeruginosa strains was very high Many bacteriological and epidemiological investigations were undertaken in order to find the sources of dissemination (mothers newborn carrier water tanks in the incubators wash bowl outlets) and links in the epidemiological chain (rubber nipples catheters suckers) The elimination of the sources and links resulted in the elimination of the Pseudomonas aeruginosa from the environment Examination of the environment showed Gram negative bacteria cultured from the objects which constituted the links in the epidemiological chain in the neonatal wards The staff was not the source of the Gram negative bacteria

The incidence of coagulase positive strains of Staphylococcus aureus was of endemic character During a period of rapid rise in incidence epidemiological investigations showed coagulase positive strains of Staphylococcus aureus in several series of powder milk

Regular co-operation of the clinicians and bacteriologists is essential in preventing neonatal aquired infections and leads to early recognition of epidemics detection and elimination of sources and links of infection and to general improvement of the organisation of the Newborns Department Infections of intrauterine origin constitute the fundamental problem in infections during the neonatal period

Ilkka Valimaki (Turku Finland) *Neonatal ECG heart rate and respiration patterns in long term recordings* (To be published in *Acta Paediat Scand*)

Antti Koivikko (Turku Finland) *Effects of metabolic acidosis on the circulation of newborns* (The presented paper represents a part of a more extensive study Cardiovascular response to hypoxia hypercapnia and metabolic acidosis *Acta Paediat Scand* Suppl *191* 1969)

Sinto Javkka (Turku Finland) *Skin temperature variation and acid bace balance during the first minutes of life*

The purpose of the present work was to determine the normal skin temperature variation and the acid bace balance in the first minutes of life

The series consisted of 76 newborn infants The skin temperatures of all the newborn were recorded and simultaneously thermovision pictures were taken From each infant a sample of cord blood was taken immediately after birth

The normal skin temperature curve is a typical one An initial rapid rise is followed by a drop in temperature The first sign of a rise after this drop occurs within 10 min after birth The mean time up to the permanent rise was 6 9 min and the mean blood pH was 7.29 In acidotic newborn the rise in temperature was slightly delayed However the delayed tem

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central regions in all children It is interesting that it is now appearing centrally before ap pearing occipitally

The latent period of the first deflection of the visually evoked potentials is shortened rapidly within the first three months of life Later on the latency is reduced only slowly until school age when the standard of the adult is reached The latency of the negative wave of a premature infant was 238 8 msec during the 34th postconceptional week At term the latency of the primary positive deflectiod amounted to 218 3 m ec and decreased during the first three months after term to 152 1 msec During school age the latent period was only 118 msec Thus *during the first three months* of life the latency is reduced by one third

The changes of the visually evoked poten tials reflect the maturation of the brain in childhood During the first three to four months of life the decisive maturation of the visual cortex takes place During this period the typical pattern of the layers of area 17 (area striata) i extending to the top of the gyri while the myelinisation is proceeding At the same time the dendrites are branching out strongly and the axosomatic synapse begin to predominate over the axodendritic synapses The morphological changes are associated with changes of the visually evoked potentials whi h thus reflect the maturation of the brain At the age of six to even years the visual cor tex is completely maturated This corresponds with the termination of the differentiation of the visually evoked potentials

The latent period of the visually evoked potentials is dependent on brain maturity and can thus be used as a very reliable method for the estimation of the conceptional age in small for date babies *The accuracy of this measurement* corresponds with the accuracy of nerve con duction velo ity determination

In children with progressing *internal* hydrocephalus already a sli_ht enlargement of the ventricles leads to a deformation of the visu ally evoked potentials The most *constant* change is the marked prolongation of the la tency which increases together with the progression of the internal hydrocephalus

The visually evoked potentials constitute a valuable complement to the diagnostic tools especially to the retinogram when di eases of the visual system are studied

E I Wallgren

perature rise was not always a sign of increased acidosis After complicated pregnancies and deliveries the final balance of thermoregulation was delayed, even though the infant s condition otherwi e appeared to be good

The authors are of the opinion that the *variation of the skin temperature of a newborn* infant reflects disturbances in the condition of the newborn and that *its measurement can be* used as a clinical aid in the evaluation of the newborn

Kalle Österlund Esko Tähti & John Lind (Helsinki Finland) *Cooling of the newborn recorded by cinethermography*

The variation of skin temperature in different parts of the newborn infants body was de monstrated in a film showing cinethermo-graphic recordings during the delivery and the *first minutes of life*

Meeting June 11 1969

H Fichsel (Bonn West Germany) *Visually evoked potentials in cerebral cortex of chil dren Development of responsiveness and changes under pathological conditions*

Evoked potentials are released in the visual cortex *by stimulation of the retina* with light flashes These potentials can be released from the inion or the occipital *regions in children of* every age and it is possible to record them from the scalp

The visually evoked potentials were studied in 256 premature and full term newborn in fants with the aid of a CAT 400 Furthermore 10 infants with internal hydrocephalus were examined

The progressive development of the visually evoked potentials beginning with the foetal stage and continuing to the end of school age is characterized by

1 *differentiation* of the number shape and sequence of the waves
2 the topographic spread of the waves
3 shortening of the latent period

Visually evoked potentials can be released in every instance during the second trimester of the foetal period Up to the eighth postcon ceptional month the visually evoked potentials consist of a primary *negative deflection fol* lowed by a positive deflection Shortly before *birth and during the newborn* period the typi cal potentials show a complex of a positive de flection followed by a negative and final posi-tive deflection From the third month of life an important differentiation takes place a nega tive wave appears in front of this complex *showing a rapidly increasing amplitude* The duration of the following large positive defle tion increases together with it s amplitude Al though the amplitude of the big positive wave then again decreases towards the end of the first year of life the duration of this defle tion increases steadily At the end of the first year of life another complex consisting of a negative and a positive wave appears proceed *ing these potentials This complex* represents the first and the second wave of the final evoked potentials At the beginning of school age the shape and the number of waves of the child s visually evoked potentials have almost attained their adult pattern

The visually evoked potentials of premature newborn infants have their *maximal intensity* over the inion They are very distinct over the *parasagital occipital* regions and can be de monstrated over parietal regions in 70 per cent of the cases and over centralregions in 15 per cent of the cases In full term newborn infants the maximum is likewise over the inion but there is less spreading of the potentials During the third month of life the maximum of the *visually evoked potentials* is changing from the inion to the occipital regions while there is hardly any spread over the non visual cortex After the sixth year of life a negative potential can again be observed as well in parietal as in

ANNOUNCEMENT

Heinz Karger Prize 1969

The prize which is awarded every year in memory of Heinz Karger the wellknown Basle publisher for an outstanding scientific work has in 1969 been conferred in equal parts to Edit Beregi Budapest and Alex Comfort London for their papers on the subject of Basic Research in Gerontology

The Heinz Karger Memorial Foundation invites the submission of papers on the following subjects

1970 An original paper on *Computers in Medical Diagnosis*

1971 An original paper on *Biochemical Aspects of Immunology*

Conditions

Manuscripts on the subject for 1970 should not exceed 30 typewritten pages and manuscripts on the subject for 1971 should not exceed 20 typewritten pages including *illustrations* tables and bibliography Manuscripts marked Competition must reach the publishers S Karger AG Arnold Bocklin Strasse 25 CH-4000 Basle 11 (Switzerland) not later than June 30 The manuscript must be typewritten on one side only double spaced and is to be submitted in duplicate and in accordance with the instructions contained in The Manuscript (Rules for the preparation of manuscripts and bibliographies of scientific papers) This booklet can be obtained free of charge from the publishers if the request is marked Competition

Language English German or French

Publication The winning papers will be published in one of the Karger journals

The award for the prize will be SFr 5000 –

The Council of the Foundation will judge the papers and confer the prizes

NEW BOOKS RECEIVED

V Dubowitz *The floppy infant* Clinics in Developmental Medicine No 31 109 pp W Heinemann Medical Books Ltd London 1969 US $6 00

D G Evans (ed) *Immunization against infectious diseases* British Medical Bulletin Vol 25 No 2 London 1969 £2

H J Kaufmann (ed) *Progress in pediatric radiology* Vol 2 *Gastro intestinal tract* 405 pp illus S Karger AG Basel New York 1969 DM 85— 170s

A Benedikt & P Csáki *Zur Frage der Entwicklung von Kindern mit niedrigem Geburtsgewicht* 167 pp Akadémiai Kiadó Budapest 1969 US $6 00

C C de Silva & N G Baptist *Tropical nutritional disorders of infants and children* 226 pp Charles C Thomas Publ Springfield Ill 1969 US $11 50

U Willenbockel *Zur Physiologie und Pathologie des Phosphatstoffwechsels* 109 pp illus Ferdinand Enke Verlag Stuttgart 1969 DM 30—

J G Howells (ed) *Modern perspectives in international child psychiatry* 878 pp Oliver & Boyd Ltd Edinburgh 1969 £8 8s

W Zublin *Chromosomale Aberrationen und Psyche* 160 pp Bibliotheca Psychiatrica et Neurologica No 140 S Karger AG Basel New York 1969 US $10 10

H L Barnett (ed) *Pediatrics—14th Edn* 1847 pp illus Butterworth & Co Ltd London 1969 £10 15s

P G Kiss *Über die Grundlagen der Menschen Psychologie* 273 pp Akadémiai Kiadó Budapest 1968 US $9 60

J Brodehl *Der renale Transport der Aminosäuren im Säuglings und Kindesalter* 97 pp illus Beihefte z Archiv f Kinderheilkunde 61 Heft Ferdinand Enke Verlag Stuttgart 1969 DM 30—

H Opitz & F Schmid (eds) *Handbuch der Kinderheilkunde* Band VIII/1 *Neurologie Psychologie Psychiatrie* F Schmid & H Asperger eds 1060 pp illus Springer Verlag Berlin Heidelberg New York 1969 DM 385—

G Masse *Croissance et développement de l'enfant à Dakar* 263 pp Centre International de L'Enfance Paris 1969 Price not given

The Cardiff Diagnostic Classification 163 pp British Paediatric Association London 1969

M M Ravitch *Repair of hernias* 189 pp illus Year Book Medical Publ Inc Chicago Ill 1969 US $10 00

Acta Pædiat Scand 59 121-126 1970

PLASMA HEMOPEXIN AND HAPTOGLOBIN IN HEMOLYTIC DISEASES OF THE NEWBORN

BENGT LUNDH FRANK A OSKI and FRANK H GARDNER

From the Hematology Research Laboratory Presbyterian University of Pennsylvania Medical Center Department of Pediatrics Hospital of the University of Pennsylvania and the Departments of Medicine and Pediatrics University of Pennsylvania School of Medicine Philadelphia Pennsylvania U.S.A

ny factors have been considered in the eval ion of hemolytic diseases of the newborn the present time it may be difficult to esti te the degree of hemolysis accurately the :ds for exchange transfusion and the effect such a transfusion Recently it has been own that the concentration of hemopexin a glycoprotein with high affinity for heme is creased in hemolytic diseases in adults and older children (10 15) Also the hemo- xin level in the normal newborn infant is out 20 of the normal adult level (3) In e present study the plasma hemopexin con ntration in a group of normal term and pre ature infants as well as newborn infants with emolytic diseases has been determined with n immunodiffusion method in an attempt to sess its value as an aid in the diagnosis of creased red cell destruction Although it is ell known that the haptoglobin level in new orn infants is generally very low (2 12 14) mmunochemical determinations of haptoglo- in were included to evaluate the relationship between these two plasma proteins in the neo- natal period

METHODS

Blood was taken by heel stab or venepuncture into a heparinized capillary tube The red cells were im mediately centrifuged the plasma portion was se parated sealed and frozen at −20 For the deter mination of hemopexin and haptoglobin the electro immunodiffusion method of Laurell was used with specific antisera for hemopexin and haptoglobin The concentration of antiserum was 0.35 and 0.10 ml 100 ml respectively and 4.5 µl of plasma diluted 1 5 with physiological saline was applied to the plate The separation time was 4 hours The height of the peaks was read with the aid of a magnifying lens graded in 0.1 mm To obtain a standard curve a serum pool from 15 healthy male blood donors was used A daily sample of the pool was thawed and diluted 1 10 1 20 1 40 and 1 80 with saline These dilutions were then applied in duplicate on every plate This serum pool was calibrated with the aid of a standardized human serum (Behringwerke) for hemopexin and by the method of Tarukoski for haptoglobin The concentration of hemopexin in the pool was found to be 63 mg/100 ml and that of haptoglobin 67 mg/100 ml given as hemoglobin bind ing capacity (HbBC) In the following the haptoglo bin results are given in HbBC All determinations were made in duplicate The variation coefficient of the method was calculated on the basis of 22 double determinations performed on the same plate For he mopexin it was found to be 6 of a mean concen tration of 26 mg/100 ml and for haptoglobin 9 of a mean concentration of 21 mg/100 ml In a material of 12 samples with a mean hemopexin and hapto globin concentration of 4.9 and 8.2 mg/100 ml res pectively the variation coefficient of the method was found to be 8% for the hemopexin and 7% for the haptoglobin method

With the methods used a comparison was made between hemopexin and haptoglobin levels in hepari nized plasma and serum respectively in varying di lutions from two healthy adults No significant dif ference could be demonstrated The methods were tested in a group of 24 healthy male blood donors aged 18-25 years The mean hemopexin level wa

The antisera were kindly supplied by professor C B Laurell Dept of Clin Chemistry Malmo General Hospital Malmo Sweden

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PLASMA HEMOPEXIN AND HAPTOGLOBIN IN HEMOLYTIC DISEASES OF THE NEWBORN

BENGT LUNDH FRANK A OSKI and FRANK H GARDNER

From the Hematology Research Laboratory Presbyterian University of Pennsylvania Medical Center Department of Pediatrics Hospital of the University of Pennsylvania and the Departments of Medicine and Pediatrics University of Pennsylvania School of Medicine Philadelphia Pennsylvania USA

ry factors have been considered in the eval on of hemolytic diseases of the newborn the present time it may be difficult to esti e the degree of hemolysis accurately the ds for exchange transfusion and the effect such a transfusion Recently it has been wn that the concentration of hemopexin a glycoprotein with high affinity for heme is creased in hemolytic diseases in adults and older children (10 15) Also the hemo- in level in the normal newborn infant is out 20 of the normal adult level (3) In present study the plasma hemopexin con ntration in a group of normal term and pre ature infants as well as newborn infants with molytic diseases has been determined with immunodiffusion method in an attempt to sess its value as an aid in the diagnosis of creased red cell destruction Although it is ell known that the haptoglobin level in new orn infants is generally very low (2 12 14) nmunochemical determinations of haptoglo- in were included to evaluate the relationship etween these two plasma proteins in the neo- atal period

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With the methods used a comparison was made between hemopexin and haptoglobin levels in hepari nized plasma and serum respectively in varying di lutions from two healthy adults No significant dif ference could be demonstrated The methods were tested in a group of 24 healthy male blood donors aged 18–5 years The mean hemopexin level was

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a statistically probably significant ($0.05 > p > 0.01$)

Fifteen of the 39 term infants had no demonstrable haptoglobin while in the remainder of the group values as high as 70 mg/100 ml were seen The mean concentration was 13 mg/100 ml (medium 12 mg/100 ml) and the standard deviation 17.4 mg/100 ml Among the 27 premature infants 19 had no demonstrable haptoglobin while among the others values up to 26 mg/100 ml were observed The mean was 3 mg/100 ml the median 0 mg/100 ml and the standard deviation 6.1 mg/100 ml

The material of term infants was further more divided into two groups one consisting of cases with demonstrable haptoglobin in plasma and the other with no haptoglobin in plasma The hemopexin level in the first group was 23 ± 7.8 mg/100 ml (mean ± 1 S.D.) and in the second group 17 ± 8.3 mg/100 ml The difference was probably significant ($0.05 > p > 0.01$)

Of eight infants in group A one of which was premature with frank hemolysis explained by either a positive Coombs test or G6PD deficiency the hemopexin level was low and varied between 4 and 16 mg/100 ml Six of these children had no haptoglobin demonstrable while two had haptoglobin levels of 7 and 12 mg/100 ml respectively (Fig 2) In group B with 4 term and one premature infant with weakly positive Coombs test or G6PD deficiency but without signs of frank hemolysis the hemopexin level was normal and varied between 4 and 28 mg/100 ml with the one low value seen in the premature infant Haptoglobin could be demonstrated in all these children in concentrations varying between 4 and 25 mg/100 ml (Fig 2) In group C composed of infants with G6PD deficiency and bilirubin above 8 mg 100 ml but without reticulocytosis or other signs of hemolysis the hemopexin levels were within the normal range and in two of the three cases haptoglobin could be found in relatively high concentration (Fig 2)

Of the four patients studied before and for varying periods after exchange transfusions

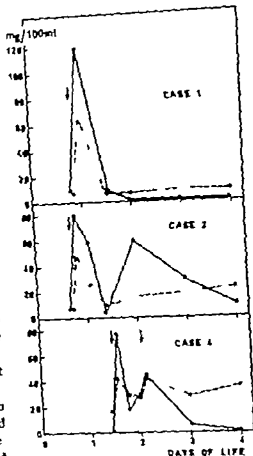


Fig 3 Serial determinations of hemopexin (- - -) and haptoglobin (——) in three infants (cases 1 2 and 4) treated with exchange transfusion (s) due to maternal isoimmunization The transfusions are indicated by arrows

due to maternal isoimmunization (Fig 3) three (cases 1 2 and 3) had an initial hemopexin level of 6 mg/100 ml Immediately after the exchange transfusion substantial increases varying from 40 to 66 mg/100 ml were observed Two of the cases thereafter showed a rapid decrease to the pretransfusion level followed by a slow increase during the following days Case 4 initially had a normal hemopexin level of 16 mg/100 ml with a moderate increase after the transfusion to 43 mg/100 ml and a decrease to 27 mg/100 ml 14 hours later A second exchange transfusion slightly increased the level to 42 mg/100 ml whereafter the concentration stabilized at approximately 30 mg/100 ml

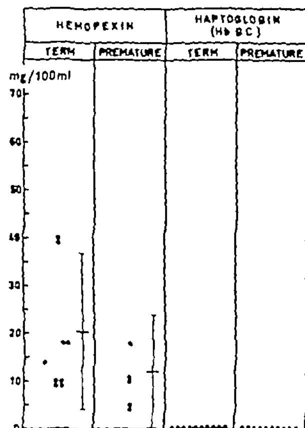


Fig 1 The plasma concentration of hemopexin and haptoglobin in 39 term and 27 premature infants on the first day of life To the right of the hemopexin results the means ±2 S D are indicated

found to be 73 mg/100 ml with a standard deviation of 9 mg/100 ml and the mean haptoglobin level 88 mg/100 ml with a standard deviation of 37 mg 100 ml

MATERIAL

Plasma was obtained within 24 hours of the time of delivery in all but one instance Thirty nine of the newborns with a normal hemoglobin hematocrit reticulocyte count blood smear glucose 6 phosphate dehydrogenase (G6PD) activity of the red cells a negative Coombs test and a normal gestational age were considered as normal term infants A second group comprised 27 hematologically normal but premature infants The gestational age was defined according to the criteria of Koenigsberger and Usher *et al* All infants less than 38 weeks gestation were classified as premature The remainder of the material was divided into three groups

A Term and premature newborns with positive Coombs test (7 patients) or G6PD deficiency (1 patient) and with reticulocytosis > 6 and a bilirubin > 8 mg/100 ml on the third day of life

B Term and premature newborns with (usually weakly) positive Coombs test (2 patients) or G6PD deficiency (3 patients) but without signs of active hemolysis (reticulocytes below 6 and bilirubin low 8 mg/100 ml on the third day of life)

C Two term and 1 premature newborn G6PD deficiency and a bilirubin of more than 8 100 ml on the third day of life but without retic cytosis or other signs of hemolysis

Finally in four cases included in group A, sa were obtained before and varying periods after change transfusions for hyperbilirubinemia secon to maternal isoimmunization In one of these c the first sample was obtained during the second of life

RESULTS

The mean plasma hemopexin level on the day of life in the 39 term infants was 31 100 ml with a standard deviation of 8 1 100 ml and a range between 8 and 42 100 ml In the 27 premature infants the m plasma hemopexin level was 12 mg/100 with a standard deviaton of 6 3 mg/100 and a range between 2 and 26 mg/100 (Fig 1) The difference between the mean he mopexin level in term and premature infants

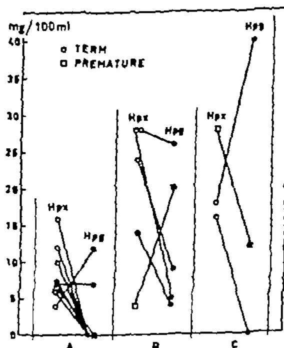


Fig 2 The concentration of hemopexin and haptoglobin in infants with positive Coombs test or G6PD deficiency The infants in group A had signs of frank hemolysis in group B no signs of hemolysis and in group C hyperbilirubinemia only o Term infants □ premature infants Hpx hemopexin Hpg hapto globin

high values but reappraisal of their clinical charts did not show any signs of complicating disease such as infection that could be responsible for the high values. The wider fluctuations and the more rapid decrease of the haptoglobin concentration after exchange transfusions may support the hypothesis that haptoglobin acts as the first line of defense in hemolysis and that hemopexin is affected only when the haptoglobin reaches the zero level and the plasma concentration of hemoglobin and of heme increases. It is remarkable that in two of the exchange transfused cases a spontaneous increase in the haptoglobin level was seen after the initial rapid decline. This elevation possibly may be explained by a release from an immature or overloaded reticulo-endothelial system of haptoglobin or haptoglobin split products reacting with the antiserum.

In the group of 8 patients with frank hemolysis due to isoimmunization or G6PD deficiency low hemopexin levels were seen although 4 cases were within the lower limit of the normal range. Cases with positive Coombs test or with G6PD deficiency but without signs of increased hemolysis all had hemopexin values within the normal range. We have at the moment no explanation for the fact that two of the cases in group A with grossly evident hemolysis and a low hemopexin concentration had significant amounts of haptoglobin in their plasma.

Even if the results of the present study indicate that a hemopexin level below 8 mg/100 ml in a term infant is a sign of pathologically increased red cell turn over, the overlap between the groups of term infants with and without grossly evident hemolysis is big. Since this overlap is even bigger among the premature infants due to their lower normal hemopexin level, the conclusion must be drawn that there is little value in a hemopexin determination to evaluate the degree of hemolysis in the newborn.

SUMMARY

Using electroimmunodiffusion methods the concentrations of hemopexin and haptoglobin in plasma were determined in 39 newborn term infants, 27 premature infants and 16 infants with G6PD deficiency or positive Coombs test with and without signs of frank hemolysis. The hemopexin concentration in term infants was found to be 21 ± 8.1 mg/100 ml (mean ± 1 S.D.) and in premature infants 12 ± 6.3 mg/100 ml. The mean haptoglobin concentration for the term infants was 13 mg/100 ml with a range of 0–70 mg/100 ml and for the premature infants 3 mg/100 ml with a range of 0–26 mg/100 ml expressed as hemoglobin binding capacity (HbBC). Only 4 out of 8 infants with positive Coombs test or G6PD deficiency and with signs of frank hemolysis had a significantly decreased hemopexin level. In 6 of these cases no haptoglobin was demonstrable. Infants with G6PD deficiency or positive Coombs test but without signs of hemolysis had a normal hemopexin level and haptoglobin could be found in all of these samples. Immediately after exchange transfusions normal or high hemopexin values were seen with a rapid decline usually within 12 hours to subnormal values. It is concluded that due to the overlap in hemopexin concentration between children with and without grossly evident hemolysis hemopexin determinations are of little value to evaluate the degree of pathologically increased red cell turn over.

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REFERENCES

1. Augener W. Immunanalyse von Glykoproteinen. In H. Peeters (ed.) *Protides of the Biological Fluids*. Proc. 12th Coll. Elsevier, Amsterdam 1964, p. 363.
2. Bergstrand C. G., Czar B. & Tarukoski P. H. Serum haptoglobin in infancy. *Scand J Clin Lab Invest 13*: 576, 1961.
3. Hanstein A. & Muller-Eberhard U. Concentrations of serum hemopexin in healthy children and adults and those with a variety of hematological disorders. *J Lab Clin Med 71*: 232, 1968.

The haptoglobin concentration parallelled the hemopexin concentration in all these cases although the fluctuations were greater A sharp fall to very low levels occurred within 24 hours of the exchange transfusion In one case the haptoglobin concentration remained at this low level while one of the remaining two cases showed a marked and the other a moderate spontaneous increase in the haptoglobin level

DISCUSSION

The presence of a heme binding protein among the β globulins has been known for many years (11) In 1960 Nyman showed that only free heme and not hemoglobin could be bound to this protein Some years later the protein was isolated and given the name of hemopexin and it could be shown that each molecule could bind one molecule of heme (6) The isolation of the protein also meant that specific antisera could be produced to allow quantitation of the protein with different types of immunochemical methods Using the radial immunodiffusion technique Augener and Muller Eberhard *et al* defined the normal range of hemopexin in different age periods indicating that newborn infants have a hemopexin concentration of approximately 20% of the adult concentration of 50–100 mg/100 ml Using different methods Sears and Muller Eberhard *et al* tabulated depressed hemopexin levels in diseases with increased red cell turnover in adults and older children The data of Sears provided support for a theory that the hemopexin level is not markedly depressed until the haptoglobin pool of plasma is depleted Some support for this theory is obtained also from the data of Muller Eberhard *et al* In order to better elucidate the relationship between these two proteins immunochemical methods also should be used for the haptoglobin determination since peroxidase methods are very inaccurate at these low levels (8)

With the method used in this study the hemopexin range in a group of healthy young adults was slightly lower than that in the study of Muller Eberhard *et al* In spite of this fact the mean value and the range for newborn term infants was slightly higher This difference may be explained in the present study by the division of the newborn material into term and premature infants This separation demonstrated a significantly lower hemopexin level among the premature infants compared to the term infants

The lower values seen in premature and term infants compared to adults may be due either to a lower rate of synthesis or a higher rate of removal If the hypothesis of Sears that the hemopexin level will not be markedly depressed until all haptoglobin is consumed is correct and if the low hemopexin level seen in hematologically normal newborn infants really was related to consumption secondary to their high red cell turnover then the hemopexin level in the group without haptoglobin should be much lower than in the group with haptoglobin in plasma The difference however was slight even though statistically significant This observation together with the circumstance that premature infants had a significantly lower hemopexin level than term infants suggests that the low hemopexin level in hematologically normal infants is primarly a consequence of an immature low synthesis

If it could be assumed that an exchange transfusion was 100% effective then the fall in the hemopexin level after that procedure should provide some information about the normal turnover rate of the protein Studies with the aid of serial haptoglobin (4) or carbon monoxide production determinations (9) respectively have shown continuing increased red cell destruction after exchange transfusions Thus the rapid fall from an increased hemopexin level to a decreased value seen in two of our patients cannot be used in the evaluation of the normal turnover rate

It has long been known that haptoglobin is absent or present in only small amounts in newborn infants (2 12, 14) and the results of the present study are consistent with this earlier data Some of the infants had rather

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THE BLEEDING DISORDER IN HEPATOMEGALIC FORMS OF GLYCOGEN STORAGE DISEASE

INGA MARIE NILSSON and P A OCKERMAN

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A bleeding tendency was found already in the first patients described with glycogen storage disease (GSD) (45 47) Bleeding has since then been seen as a common symptom in patients with hepatomegalic forms of GSD (types I III and VI)

A more detailed study of the nature of the bleeding disorder has been made by only a few authors French and German authors partly in collaboration (1 18–21) suggested that the bleeding in GSD type I was caused by a thrombopathy due to a deficiency of glucose 6-phosphatase and a storage of large amounts of glycogen in the thrombocytes In patients with GSD type III and VI no thrombopathy was found (1) Gilchrist *et al* (13) in a study of eight cases of GSD type I also found a thrombopathy with prolonged bleeding time and reduction in platelet adhesiveness and availability of platelet factor 3

The existence of a specific glucose 6 phosphatase in normal thrombocytes has however not been verified by other authors (17 35 43) and the cause of the thrombopathy in GSD type I may therefore need reinvestigation

The hemostatic defect in GSD type III and VI has not been studied in any detail It is obvious that the hemostatic defect in these types of GSD cannot be explained by a deficiency of glucose 6-phosphatase in the thrombocytes since in these types lack of glucose 6 phosphatase is not the primary deficiency in the liver

These considerations formed the basis for the present investigation of the bleeding disorder in patients with the hepatomegalic types I III and VI of GSD

MATERIAL

Patients with Glycogen Storage Disease

Type I (glucose-6 phosphatase deficiency)

Case 1 D T Female 23 She has never had any definite bleeding symptoms

Case 2 A T Male 21 He often had nose bleeding from early childhood but after puberty this tendency disappeared Surgical liver biopsy at age 12 was followed by profuse bleeding

Case 3 K T Female 17 This patient often has had nose bleeding from early childhood This tendency is still remaining She also has profuse menstruation Surgical liver biopsy at age 8 was followed by profuse bleeding

Case 4 S T Male 9 A slight tendency to nose bleeding started in early childhood

Cases 1–4 are siblings and have been studied in great detail (4 31 36) There remains no doubt about the diagnosis

Case 5 E I L Female 11 Published as case 7 in ref 34 Since early childhood she has a very marked bleeding tendency in the form of nose bleeding bruising and prolonged bleeding after cuts Because of this no enzymatic studies on liver tissue could be done The diagnosis GSD type I nevertheless is quite clear because of the findings made in her sister (case 6)

Case 6 H L Female 1 sister of case 5 Published as case 4 in ref 22 Glycogen and enzyme assays on liver tissue support the diagnosis She has no bleeding tendency

Type III (Amylo 1 6 glucosidase deficiency)

Case 7 A L Female 12 Published as case 13 in ref 34 Glycogen and enzyme assays on muscle tissue

4 Heide K Haupt H Stonko K & Schultze H E On the heme binding capacity of hemopexin *Clin Chim Acta 10* 460 1964
5 Kauder E & Mauer A M Hemolysis as a contributing factor in the bilirubin rebound after exchange transfusion *J Pediat 60* 163 1962
6 Koenigsberger M R Judgement of foetal age I Neurologic evaluation *Pediat Clin N Amer 13* 835 1966
7 Laurell C B Quantitative estimation of proteins by electrophoresis in agarose gel containing Antibodies *Anal Biochem 15* 45 1966
8 Laurell C B & Gronwall C Haptoglobins *Advances Clin Chem 5* 135 1962
9 Maisels M J Pathak A & Nelson N M Carbon monoxide production and bilirubin excretion in normal and erythroblastotic newborn infants *Prog Society for Pediatric Res 39th* Annual Meeting 43 1969 (abstr)
10 Muller Eberhard U Javid J Liem H H Hanstein A & Hanna M Plasma concentrations of hemopexin haptoglobin and heme in patients with various hemolytic diseases *Blood 32* 811 1968
11 Neale F C Aber G M & Northam B E The demonstration of intravascular hemolysis by means of serum paper electrophoresis and a modification of Schumm s reaction *J Clin Path 11* 206 1958
12 Nyman M Serum haptoglobin Methodological and clinical studies *Scand J Clin Lab Invest 11* (Suppl 39) 1 1959
13 Nyman M On plasma proteins with heme or hemoglobin binding capacity *Scand J Clin Lab Invest 12* 121 1960
14 Rausen A R Gerald P S & Diamond L K Haptoglobin patterns in cord blood serum *Nature 191* 717 1961
15 Sears D A Plasma heme binding in patients with hemolytic disorders *J Lab Clin Med 71* 484 1968
16 Tarukoski P H Quantitative spectrophotometric determination of haptoglobin *Scand J Clin Lab Invest 18* 80 1966
17 Usher R McLean F & Scott K E Judgement of foetal age II Clinical significance of gestational age and an objective method for its assessment *Pediat Clin N Amer 13* 823 1966

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Key words Newborn infants hemolysis hemopexin haptoglobin

Table 1 *Bleeding and coagulation tests in patients with glycogen storage disease*

Abnormal values are underlined

	Case 1 (No of invest −?)	Case (No of invest)	Case 3 (No of invest −2)	Case 4 (No of invest −2)	Case 5 (No of invest −2)	Case 6 (No of invest −2)	Case 7	Case 8	Case 9 (No of invest −4)	Case 10	Normal range
Bleeding time											
Duke min	4	2.5	2	4–8	1	4	3	4	2–8	4	1–4
Ivy min	9	18	30	14–	> 30	11	9	4–15	20	10	6–15
Coagulation time											
glass min	14	18	14	14	12	9	—	18	13	12	8–14
plastic min	25	3	8	4	40	20	—	22	21	19	15–25
Prothrombin consumption	2	12	12	25	21	—	—	—	0	—	0–30
Platelet number per mm^{3}	160 000	140 000	180 000	246 000	340 000	90 000	10 000	240 000	290 000	450 000	135 000–300 000
Platelet adhesiveness											
Hellem's whole blood	34–40	29–40	5–34	40–4	30–32	1 4	34	34	1–17	31 34	28–40
Salzman's method	4–17	26	23	1	3	—	0	—	0–5	—	20–60
Platelet factor 3 availability	45	5	—	—	—	—	—	—	100–130	—	90–190
Recalcification time sec	143	172	120	143	10	166	100	130	10–190	140	110–180
One-stage prothrombin time sec	14	14	16	16	15	15	15	14	15	15	14–16
P & P	100	112	140	18	00	13	64	111	110–190	116	80–120
Prothrombin immunochem	—	—	139	15	00	—	—	—	17	—	80–120
Factor V	10	85	90	97	96	14	18	177	99–135	148	80–120
Factor VIII	160	103	138	137	65	73	04	129	125	88	60–140
Factor IX	—	—	150	137	96	—	—	125	148	—	60–140
Fibrinogen g/100 ml	0.55	0.8	0.48	0.35	0.41	0.40	0.35	0.76	0.41	0.34	0.26–0.34
Thrombin time (3 NIH/ml) sec	19	18	17	7	18	—	20	—	18	20	18–20
Plasminogen	139	11	157	181	95	—	102	—	144	81	60–140
– immunochem			118	132							
Urokinase inhibitors	93	83	83	75	110	—	184	—	164	—	60–140
Fibrinolytic activity (untreated lysis)											
plasma min	0	0	0	0	0	0	—	—	0	0	0–50
reagent euglob prec mm^{2}	0	10	20	25	25	25	—	—	0	70	0–70
Fibrinolytic split products mg/100 ml	0	0	0	0	0	—	0	—		—	0

support the diagnosis She has a very moderate bleeding tendency (nose bleeding bruising) not definitely pathological since early childhood

Case 8 J B Male 2 Published as case 2 in ref 22 Glycogen and enzyme assays on liver and muscle tissue support the diagnosis He has no bleeding tendency

Type VI (Liver phosphorylase deficiency)

Case 9 M B Male 6 Published as case 1 in ref 22 The diagnosis is supported by glycogen and enzyme assays on liver and muscle tissue He has a moderate bleeding tendency (nose bleeding bruising prolonged bleeding after cuts)

Case 10 T N Female 1 Published as case 3 in ref 22 The diagnosis is supported by glycogen and enzyme assays on liver tissue She has no obvious bleeding tendency

METHODS

Collection of blood The blood was collected with silicone technique and citrated plasma and serum were prepared as described previously (26 37)

Coagulation tests The following determinations were made coagulation time in glass and plastic tubes recalcification time of plasma prothrombin consumption prothrombin + factor VII + factor X (Owren s P & P test) factor V AHF (f VIII) haemophilia B factor (f IX) fibrinogen and thrombin time The methods used have been described earlier (24 27) In some patients the prothrombin content in plasma was also determined by an immunochemical method (12)

The *bleeding time* was performed with Duke s method using standardized haemolets (Dade Reagent Inc Miami Florida U S A) Determinations were performed on both ears Normal range 1 to 4 min Use was also made of the method of Ivy as modified by Borchgrevink & Waaler (3 8 28) Normal range 5 to 15 min

Platelet counts were made by the method of Bjorkman (2)

Platelet adhesiveness was measured according to a slight modification (8) of Hellem s whole blood method (15) Platelet adhesiveness was also measured with the original method of Salzman (39)

Platelet adhesion aggregation and spreading were studied by phase contrast microscopy of a drop of platelet rich citrated plasma placed on a glass slide under a cover glass Platelet aggregation after addition of connective tissue suspension or ADP was studied macroscopically microscopically and with the aid of a photometer (9)

Clot retraction was determined in diluted platelet rich plasma according to a modification of Voss method (9)

Platelet factor 3 release was determined according to Hardisty & Hutton (14)

Fibrinolytic studies The following determinations were made euglobulin clot lysis time fibrinolytic activity of plasma and resuspended euglobulin precipitate plasminogen inhibitors of plasminogen activation by urokinase α macroglobulin and fibrinolyt split products Unless otherwise stated the method described earlier were used (10 23 29 37) Plasminogen was determined both by a clot method (3[illegible] and by an immunochemical method (11)

RESULTS

The results of the coagulation studies are give in Table I *GSD type I* Cases 1–5 all betwee 9 and 23 years old had prolonged Ivy bleedin time and three of them had low values for pla telet adhesiveness according to Salzman *method There was no decrease of the platele* adhesiveness according to Hellem s method The platelet factor 3 availability was deter mined in cases 1 and 2 and was abnormall low in these cases Platelet count was norma in all except case 5 Four of the patients wit GSD type I showed increased levels of the fac tors measured in Owren s P & P test This find ing was supported by immunochemical deter mination of prothrombin The plasminoge content was increased in three of the cases Fibrinogen levels were high in all six patients The other coagulation factors were mostl within normal ranges The urokinase inhibitor were high in case 5 but normal in the othe cases There were no signs of increased fibri nolytic activity *Case 6 1 year old could no be studied in all details She had normal Duk* and Ivy bleeding time and no signs of a bleed ing disorder but she had a high number o platelets

GSD type III Cases 7 and 8 had norma values throughout with the exception of a low platelet adhesiveness according to Salzman s method in case 7 *somewhat high platelet count* in case 8 and aberrant values for a few coa gulation factors The patient in case 8 was only 2 years old

GSD type VI Case 9 6 years old showe the most marked changes in our material He had somewhat high platelet count prolonge Duke and Ivy bleeding times somewhat pro *longed coagulation time* and decreased platele adhesiveness both according to Hellem s and

Table 1. *Bleeding and coagulation tests in patients with glycogen storage disease*

Abnormal values are underlined

	Case 1 (No of in vest –[illegible])	Case 2 (No of in vest 2)	Case 3 (No of in vest –)	Case 4 (No of in vest)	Case 5 (No of in vest –2)	Case 6 (No of in est –2)	Case 7	Case 8	Case 9 (No of in vest –4)	Case 10	Normal range
Bleeding time											
Duke min	4	2.5	2	4	1	4	3	4	4	4	1–4
Ivy min	[illegible]	18–[illegible]	[illegible]0	14	> 90	11	9	4–15	> 30	18	6–15
Coagulation time											
glass min	14	15	14	14	12	9	—	15	18	12	8–14
plastic min	25	23	9	4	40	20	—	22	31	19	15–25
Prothrombin consumption	2	1	12	25	21	—	—	—	0	—	0–30
Platelet number per mm³	160 000	140 000	190 000	246 000	340 000	500 000	210 000	340 000	380 000	480 000	135 000–300 000
Platelet adhesiveness											
Hellem's whole blood	34–40	29–40	[illegible]–34	40–4[illegible]	30 32	1 3	34	34	1 17	31 34	28–40
Salzman's method	4 11	76	3	1	3	—	0	—	0–8	—	20 60
Platelet factor 3 availability	95	5	—	—	—	—	—	—	100 130	—	90–150
Recalcification time sec	143	172	1[illegible]0	143	16	166	100	130	1[illegible]0 180	140	1[illegible]0 180
One-stage prothrombin time sec	14	14	16	16	15	15	15	14	15	15	14–16
P & P	100	112	140	18	[illegible]	1[illegible]	44	111	1[illegible]–180	116	80–120
Prothrombin immunochem	—	—	139	18	[illegible]00	—	—	—	77	—	80–120
Factor V	70	85	90	97	96	1[illegible]4	1[illegible]8	1[illegible]7	99–125	148	80–170
Factor VIII	100	187	138	137	65	73	04	129	175	88	60–140
Factor IX	—	—	180	137	96	—	—	125	149	—	60–140
Fibrinogen g/100 ml	0.35	0.[illegible]	0.48	0.32	0.43	0.40	0.35	0.76	0.41	0.34	0.[illegible]–0.34
Thrombin time (3 NIH/ml) sec	19	18	17	17	18	—	20	—	18	20	18–20
Plasminogen	139	11	189	181	95	—	100	—	144	81	60–140
– immunochem			178	13							
Urokinase inhibitors	93	83	83	75	390	—	184	—	184	—	60–140
Fibrinolytic activity on untreated plates											
plasma mm²	0	0	0	0	0	0	—	—	0	0	0–50
resusp euglob prec mm	0	10	0	25	5	25	—	—	0	[illegible]0	0–70
Fibrinolytic split products mg/100 ml	0	0	0	0	0	—	0	—		—	0

Salzman's method At examination under the phase contrast microscope his platelets showed a definitely weak aggregation The platelet factor 3-availability test however on repeated occasions showed normal values This patient also had a high level of prothrombin and fibrinogen Case 10 one year-old, had normal Ivy bleeding time and normal platelet aggregation, but a high platelet count

All patients The following values are not included in the table Clot retraction was normal in all the patients On direct microscopical examination the platelets appeared normal in all cases At examination under the phase contrast microscope the platelets adhered to glass and aggregation occurred But the spontaneous aggregation was delayed in cases 1 2, 3 4 5 and 9 Spontaneous aggregation was normal in cases 8 and 10 In cases 6 and 7 no such investigation was performed In all cases studied the platelets aggregated after addition of ADP and connective tissue suspension

DISCUSSION

Bleeding tendency (44 46) prolonged bleeding time (43) and high humber of thrombocytes (40) were the first abnormalities of the hemostatic system found in patients with GSD No detailed explanation of the nature of the abnormality could be given by these early authors Schwartz *et al* (40) found the consumption of prothrombin to be at the lower limit of the normal Sidbury (41) thoroughly investigated several patients for impaired circulating clotting factors but found no abnormalities This author therefore considered the bleeding tendency to be caused by altered metabolism on the arterioles and veins resulting in delayed or ineffective constriction after injury Van Creveld (45) found a deficiency of variable clotting factors, sometimes also in the parents

A qualitative defect of thrombocytes in patients with GSD was first demonstrated by Lelong *et al* (17) In a more detailed study of six patients with GSD type I by this French group (1) was demonstrated abnormalities of the bleeding time (prolonged) number of platelets (increased) prothrombin consumption (decreased) thromboelastogram (moderate hypocoagulability) and thrombocyte thromboplastin generation (retarded) Normal findings were reported for capillary resistance, clot retraction coagulation time and heparin tolerance In collaboration with German authors (19 20) it was shown that patients with GSD type I have very high levels of glycogen and a deficiency of glucose 6 phosphatase in the thrombocytes The conclusion was drawn that these findings might explain the thrombopathy in GSD type I In four patients with GSD type III and one patient with GSD type VI essentially normal findings were reported except for a high number of thrombocytes It was concluded that patients with GSD type III and VI did not have a thrombopathy similar to that in GSD type I

Later authors have put in doubt the existence of a specific glucose 6 phosphatase in normal thrombocytes (16 34 42) Also no endoplasmatic reticulum, the structure to which specific glucose 6 phosphatase is firmly bound could be found in thrombocytes (16)

An increased amount of glycogen in the thrombocytes has been reported not only in GSD type I but also in several other disorders e g Glanzmann's thrombasthenia, von Willebrandt's disease certain malignant and essential thrombocythemias and certain idiopathic chronic thrombocythopenias (references in Alagille *et al*) (1)

Recently Gilchrist *et al* (13) published an investigation of eight patients with GSD type I Five of these (age 12–20) had a bleeding tendency while three (age 1–2) had not A high platelet count was found in three of the patients including two of those under 2 years of age In the five patients over 12 years of age a prolonged bleeding time (according to Ivy's method) and a decreased availability of platelet factor 3 was found A significant reduction in platelet adhesiveness was found *in all eight* patients Glucose infusion or transfusion of normal platelets did not normalize thrombocyte

adhesiveness The conclusion drawn by Gilchrist *et al* was that "although an intracellular defect may account for the abnormal platelet function environmental plasma factors appear to play a role in the development of the platelet lesion

Many authors describe a bleeding tendency in their patients already from birth (1 21 38 45) Sidbury (41) in contrast to this considers that the bleeding tendency rarely appears before one year of age but after that age it is common especially in type I but also in type III We believe that all authors may be right the bleeding tendency sometimes beginning very early and in other cases appearing only after a year or two Alagille *et al* (1) had a few patients with no platelet abnormality during their first year of life but with a definite such abnormality later

In our investigation of 6 patients with GSD of type I we found an abnormal platelet function in 5 of them The one with normal values was a one year old girl All these patients had a normal platelet adhesiveness according to Hellem's method but decreased according to Salzman's method *It is possible that* the values obtained with the Hellem's method are falsely high *in these* patients All these patients had a very marked lipaemia Cronberg & Nilsson (6) have found that infusion of Intralipid in normal individuals causes a marked increase of the platelet adhesiveness as measured by Hellem's method but has no effect on the platelet adhesiveness as measured by Salzman's method In patients with thrombasthenia Intralipid also caused an increase of the adhesiveness assayed by Hellem's method but Intralipid had no effect on the prolonged bleeding time or the bleeding tendency The change observed in platelet adhesiveness was therefore believed to be an artifact with no clinical relevance It was thought that the fat droplets might have changed the properties of the glass surface with consequently increased adhesion of the platelets The findings of normal platelet count normal clot retraction normal Duke bleeding time prolonged Ivy bleeding time abnormal platelet factor 3 release decreased platelet adhesiveness (Salzman) delayed spontaneous platelet aggregation but normal aggregation after addition of connective tissue suspension thrombin and ADP are similar to those found in patients with mild thrombasthenia (7 9) Our results concerning the platelet abnormality in GSD type I agree largely with those reported by Gilchrist et al (13)

Of interest was the finding that 4 of the 6 patients had increased levels of prothrombin and plasminogen determined both by clot methods and by immunochemical methods From the report of Gilchrist *et al* it is clear that 5 of their 8 patients had *somewhat high* prothrombin level determined by Quick's method These authors did not comment upon this Lipaemia does not cause increased prothrombin and plasminogen levels (6 25)

We investigated only two patients with GSD of type III and one of these patients was only 2 years old *Both of them had* normal values throughout with the exception of a decreased platelet adhesiveness according to Salzman's method in the older girl It is known however that even normal individuals can have a low value according to Salzman (5 39) Thus no definite platelet abnormality was demonstrated in GSD of type III These patients had neither any bleeding symptoms Alagille *et al* (1) found no thrombopathy in patients with GSD *of type III* but they did not study the platelet adhesiveness or platel t aggregation Our patients *with* GSD of type III had normal prothrombin levels

Alagille *et al* (1) also reported that there was no thrombopathy in GSD of type VI Of our two patients with GSD type VI the one year old boy was completely normal but the 6 years old boy had a marked prolongation of bleeding time and a significant reduction of platelet adhesiveness It appears probable that our patient with GSD of type VI had the same type of bleeding disorder as the ones with GSD of type I The patient with GSD type VI had also a high prothrombin level

We have thus found that patients with GSD of type I and VI have a bleeding disorder which is most similar to that described in patients with mild and/or moderately severe thrombasthenia (7) In the patients that were 1 and 2 years old no definite changes were observed The biochemical basis for the platelet lesion is unknown

The practical implications of our findings are important It is clear that a patient may appear normal in his coagulation bleeding status if only such simple tests are performed as bleeding time according to Duke platelet count and coagulation time in glass tubes or on a watch glass and if a thorough history is not taken Nevertheless the patient may have a bleeding disorder When operations are necessary or in accidental bleeding fresh thrombocyte rich blood taken with silicone or plastic technique or thrombocyte suspensions constitute the best available remedies to normalize the bleeding tendency The blood substitute Macrodex must not be used since it would increase the bleeding tendency

SUMMARY

The bleeding disorder in glycogen storage disease was studied in six patients with type I and two patients each of type III and VI The most commonly seen abnormalities were prolonged Ivy bleeding time low values for platelet adhesiveness increased number of platelets and high values for prothrombin and fibrinogen It is concluded that patients with type I and VI and possibly also type III may have a bleeding disorder similar to thrombasthenia When operations are necessary or in accidental bleeding in patients with glycogen storage disease fresh thrombocyte rich blood or thrombocyte suspensions may be needed to normalize the bleeding tendency The blood substitute Macrodex must not be used since it would increase the bleeding tendency

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REFERENCES

1 Alagille D Gabilan J C & Leloet M Les anomalies plaquettaires au cours de glycogenoses hépatiques Etude de 11 cas *Helv Paediat Acta 18* 120 1963
2 Bjorkman S E A new method for enumeration of platelets *Acta Haemat 22* 377 1959
3 Borchgrevink C F & Waaler B A The secondary bleeding time A new method for the differentiation of hemorrhagic diseases *Acta Med Scand 162* 361 1958
4 Brante G Kaijser K & Öckerman P A Glycogenosis type I (lack of glucose 6 phosphatase) in four siblings *Acta Paediat Scand* Suppl *157* 1964
5 Cronberg S Investigations in haemorrhagic disorders with prolonged bleeding time but normal number of platelets with special reference to platelet adhesiveness *Acta Med Scand* Suppl *486* 1968
6 Cronberg S & Nilsson I M Coagulation studies after administration of a fat emulsion Intralipid® *Thrombos Diathes Haemorrh 18* 664 1967
7 — Investigation of patients with mild thrombasthenia—a haemorrhagic disorder with prolonged bleeding time probably due to a primary platelet defect *Acta Med Scand 183* 163 1968
8 Cronberg S Nilsson I M & Silwer J Studies on the platelet adhesiveness in von Willebrand's disease *Acta Med Scand 180* 43 1966
9 Cronberg S Nilsson I M & Zetterqvist E Investigation of a family with members with both severe and mild degree of thrombasthenia *Acta Paediat Scand 56* 189 1967
10 Ganrot P O Determination of α_2-macroglobulin as trypsin protein esterase *Clin Chim Acta 14* 493 1966
11 Ganrot P O & Nilehn J E Immunochemical determination of human plasminogen *Clin Chim Acta 22* 335 1968
12 — Immunochemical determination of human prothrombin *Scand J Clin Lab Invest* 21 238 1968
13 Gilchrist G S Fine R N & Donnell G N The hemostatic defect in glycogen storage disease type I *Acta Paediat Scand 57* 205 1969
14 Hardisty R M & Hutton R A The kaolin clotting time of platelet rich plasma A test of platelet factor 3 availability *Brit J Haemat 11* 258 1965
15 Hellem A J The adhesiveness of human blood platelets in vitro *Scand J Clin Lab Invest* Suppl *51* 1960
16 Huijing F *En vermindering in het perifere bloed bij glykogeenziekte* Thesis Amsterdam 1964
17 Lelong M Alagille D Gentil C & Gabilan J C Glycogenose hepatique par déficit en phosphorylase *Rev Franç Etud Clin Biol 5* 672 1960
18 Linneweh F Fortschritte in der Erkennung heterozygoter Merkmalsträger bei erblichen Enzymopathien *Klin Wschr 40* 553 1962

19 Lassenach F Lohr G W Waller H D & Gross R Über die Diagnose von Glykogenosen der Glukose 6 Phosphatase Aktivität und dem Glykogengehalt der Thrombocyten *Enzymol Biol Clin* 188 1962
20 — Heterozygoten Test für Glykogenose (v Gierke's Krankheit) *Klin Wschr* 41 352 1963
21 Lohr G W Pathogenese und Differentialdiagnose der Glykogenosen *Deutsch Med Wschr* 90 1549 1965
2? Lundqvist A & Ockerman P A Fine needle aspiration biopsy of human liver for enzymatic diagnosis of glycogen storage disease and gargoyl *Acta Paediat Scand* 1970 in press
23 Nilehn J E Separation and estimation of split products of fibrinogen and fibrin in human serum *Thrombos Diathes Haemorrh* 18 487 1967
24 Nilehn J E & Nilsson I M Demonstration of fibrinolytic split products in human serum by an immunological method in spontaneous and induced fibrinolytic states *Scand J Haemat* 1 313 1964
25 Nilsson I M Relationen mellan fettmetabolism blodkoagulation och fibrinolys *Nervenforskning* 3 66 1960
26 Nilsson I M Blombäck M & von Francken I On an inherited autosomal hemorrhagic diathesis with antihemophilic globulin (AHG) deficiency and prolonged bleeding time *Acta Med Scand* 159 35 1957
Nilsson I M Blombäck M & Ramgren O Haemophilia in Sweden I Coagulation studies *Acta Med Scand* 170 665 1961
? Nilsson I M Magnusson S & Borchgrevink Ch The Duke and Ivy methods for determination of the bleeding time *Thrombos Diathes Haemorrh* 10 223 1963
9 Nilsson I M & Olow B Fibrinolysis induced by streptokinase in man *Acta Chir Scand* 123 247 1962
30 Nilsson I M Skanse B & Gydell K Fibrinolysis in Boeck sarcoid *Acta Med Scand* 159 463 1957
31 Ockerman P A Phosphorylated glycolytic intermediates in liver and blood cells in members of family with glycogenosis type I *Clin Chim Acta* 8 343 1963
3 — Glycerol in plasma in glycogenosis of Cori's type I *Clin Chim Acta* 8 910 1963
33 — Glucose 6 phosphatase in human jejunal mucosa Lack of activity in glycogenosis of Cori's type I *Clin Chim Acta* 9 151 1964
34 — Glycogen storage disease in Sweden *Acta Paediat Scand* Suppl 160 1965
35 — Glucose glycerol and free fatty acids in glycogen storage disease type I Blood levels in the fasting and non fasting state Effect of glucose and adrenalin administration *Clin Chim Acta* 12 370 1965
36 — *In vitro* studies of adipose tissue metabolism of glucose glycerol and free fatty acids in glycogen storage disease type I *Clin Chim Acta* 12 383 1965
37 Paraskevas M Nilsson I M & Martinsson G A method for determining serum inhibitors of plasminogen activation *Scand J Clin Lab Invest* 14 138 1962
38 Rovner A Caldera R Sarrut S & Houllemare L Les glycogénoses néonatales *Arch Franç Pédiat* 17 1 1960
39 Salzman E W Measurement of platelet adhesiveness A simple *in vitro* technique demonstrating an abnormality in von Willebrand's disease *J Lab clin Med* 62 724 1963
40 Schwartz R Ashmore J & Renold A E Galactose tolerance in glycogen storage disease *Pediatrics* 19 585 1957
41 Sidbury J B Jr The genetics of the glycogen storage diseases *in* A G Steinberg & A G Bearn (eds) *Progress in Medical Genetics* 4 Grune & Stratton New York 1965 p 32
42 Steinitz K Laboratory diagnosis of glycogen diseases *in* H Sobotka & C P Stewart (eds) *Advances in Clin Chem* 9 Academic Press, New York 1967 p 227
43 Ullrich E Die Glykogenkrankheit *Jb Kinderheilk* 137 267 1929
44 Van Creveld S Over een bijzondere stoornis in de koolhydraatstofwisseling in den Kinderleeftijd *Nederl Maandschr Geneeskunde* 15 349 1928
45 — The clinical course of glycogen disease *Canad Med Ass J* 88 1 1963
46 Von Gierke E Hepato-nephromegalia glykogenica (Glykogenspeicherkrankheit der Leber und Nieren) *Beitr path Anat* 82 497 1929

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SUMMARY

To determine whether intrauterine infections play any part in the aetiology of spina bifida cystica, the immunoglobulin gamma M levels of cord blood from affected children were compared with matched controls No difference was found It is concluded that infection does not play a significant role in the aetiology of spina bifida cystica

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REFERENCES

1 Alford C A Schaffer J Blankinship P W J Strumfjord J V & Cassady G A correlative immunologic microbiologic and clinical approach to the diagnosis of acute and chronic infections in newborn infants *New Eng J Med 277* 437 1967
2 Bernstein L & Weatherall M *Statistics for Medical and Other Biological Studies* E & S Livingstone Edinburgh and London 1952
3 Buckley R H Dees S C & O Fallow W M Serum immunoglobulins—Levels in normal children and in uncomplicated childhood allergy *Pediatrics 41* 600 1968
4 Carter C O Laurence K M & David P A The genetics of the major central nervous system malformations based on the South Wales Socio genetic Investigation *Devel Med Child Neurol* 13 30 1967
5 Eckstein H B Myelomeningocele basic considerations *Hospital Medicine 2* 896 1968
6 Eckstein H B & MacNab G H Myelomeningocele and hydrocephalus The impact of modern treatment *Lacet I* 842 1966
7 Gitlin D The differentiation and maturation of specific immune mechanisms *Acta Paediat Scand 172* 60 1967
8 Lundstrom R Rubella during pregnancy *Acta Paediat Scand 51* 133 1962
9 Mainland D *Elementary Medical Statistics* W B Saunders London and Philadelphia 1952
10 Mancini G Carbonara A O & Heremans J F Immunochemical quantitation of antigens by single radial immunodiffusion *Immunochemistry 2* 235 1960
11 Manson M M Logan W P D & Loy *Rubella and Other Virus Infections during Pregnancy*—Reports on Public Health and Medical Subjects Her Majesty s Stationary Office 101 Ministry of Health London 1960
12 Remington J S Millar M J & Brownlee I IgM antibodies in acute toxoplasmosis I Diagnostic significance in congenital cases and a method for their rapid demonstration *Pediatrics 41 1082 1960*
13 Scotti A T & Logan L A specific IgM antibody test in neonatal congenital syphilis *J Pediat 73* 242 1968
14 Sever J & Berendes H W Cord blood gamma M as a screening test for congenital viral infections *The Soc Paed Res* 37th Annual Meeting Pennsylvania 1967
15 Sieber O F Jr Fulginiti V A Brazie J & Umlauf H J Jr In utero infection of the fetus by herpes simplex virus *J Pediat 69* 30 1966
16 Soothill J F Hayes K & Dudgeon J A Immunoglobulins in congenital rubella *Lancet I* 1385 1966
17 St Geme J W Jr & Noren G R Proposed embryopathic relation between mumps virus and primary endocardial fibroelastosis *New Eng J Med 275* 339 1966
18 Stiehm R E Ammann A J & Cherry J D Elevated cord macroglobulins in the diagnosis of intrauterine infections *New Eng J Med 275* 971 1966
19 Stoller A & Collmann R D Incidence of infectious hepatitis followed by Down s syndrome nine months later *Lancet II* 1221 1965
20 Weller T H & Hanshaw J B Virological and clinical observations on cytomegalic inclusion disease *New Eng J Med* 266 1233 1962
21 West C D Hong R & Holland N H Immunoglobulin levels from the newborn period to adulthood and in immunoglobulin deficiency states *J Clinic Invest 41* 2054 1962
22 van Furth R Schmit H M E & Hymans W Immunologic development of the human fetus *J Exp Med 122* 1173 1965

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URINARY TESTOSTERONE EXCRETION IN CHILDREN WITH CONGENITAL ADRENAL HYPERPLASIA

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Urinary testosterone excretion in untreated children with congenital adrenal hyperplasia has been reported in a few studies (3 4 11 15 16) In only one instance the study was extended into the newborn period by including a four week-old girl(3)

In the present study four newborn girls and three older patients suffering from congenital adrenal hyperplasia were investigated The purpose of this study was to see if possible relationships existed between the level of urinary testosterone excretion and indices of virilisation such as fusion of the labia minora in newborn girls and advancement of skeletal maturation in boys

alongside the original sample The recovery of this sample ranging from 36%–43% was used to correct for losses in the original sample

One twentieth of the 24-hour urine volume was hydrolysed with beta glucuronidase and extracted with ether—ethyl acetate (2 1) 11-oxy and 11 deoxy 17 ketosteroids were determined spectrophotometrically after paper chromatography in the system light petroleum—toluene—85% acetic acid (1 1 2) (2) Part of the fraction of medium polarity containing pregnanetriol was oxidised with sodium bismuthate and then chromatographed in the system—light petroleum—dekalin—85% methanol (1 1 2) (2) The Zimmermann chromogen with mobility similar to authentic aetiocholanolone was eluted and measured spectrophotometrically (6) After subsequent solvolysis of the spent urine 11-deoxy 17 ketosteroids (sulphate fraction) were also estimated

Skeletal maturation was assessed according to Greulich & Pyle (7) and fusion of the labia minora according to Prader (12)

METHODS

For the determination of testosterone one tenth of the 24 hour urine collection was hydrolysed with beta glucuronidase and extracted with ether Chromatography in the systems benzene—ethyl acetate (1 1) (9) light petroleum—dekalin—85% methanol (2 1 1) (?) was followed by acetylation and further chromatography in the system benzene—ethyl acetate (3 1) (9) Androstenedione was separated from testosterone on the first thin layer plate (9) and epitestosterone was well separated in the paper chromatographic step (4) The final step of the formation of an acetylated product increases the specificity of the method and the separation of the acetylated testosterone from the other products is successfully achieved in the second thin layer step (9)

The amount of testosterone acetate was determined fluorometrically employing testosterone acetate for the standard curve 10 μg of testosterone was added to a duplicate urine aliquot and processed

PATIENTS

All seven patients were diagnosed as suffering from congenital adrenal hyperplasia due to 21 hydroxylase deficiency on the basis of clinical and biochemical findings The patients were normotensive and had not received steroid therapy when they were first seen Three of the four newborn girls had the salt losing variant of the condition The three older patients had the non-salt losing variant of the disease (Table 1)

RESULTS

In the three newborn girls with the salt losing condition the degree of labial fusion corresponded with the level of urinary testosterone excretion (Table 1) However the patient with the smallest degree of labial fusion was studied at the age of 21 days in contrast to the other

SUMMARY

To determine whether intrauterine infections play any part in the aetiology of spina bifida cystica, the immunoglobulin gamma M levels of cord blood from affected children were compared with matched controls No difference was found It is concluded that infection does not play a significant role in the aetiology of spina bifida cystica

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REFERENCES

1 Alford C A Schaffer J Blankinship P W J Strumfjord J V & Cassady G A correlative immunologic microbiologic and clinical approach to the diagnosis of acute and chronic infections in newborn infants *New Eng J Med 277* 437 1967

2 Bernstein L & Weatherall M *Statistics for Medical and Other Biological Studies* E & S Livingstone Edinburgh and London 1952

3 Buckley R H Dees S C & O Fallow W M Serum immunoglobulins—Levels in normal children and in uncomplicated childhood allergy *Pediatrics 41* 600 1968

4 Carter C O Laurence K M & David P A The genetics of the major central nervous system malformations based on the South Wales Socio genetic Investigation *Devel Med Child Neurol 13* 30 1967

5 Eckstein H B Myelomeningocele basic considerations *Hospital Medicine 2* 896 1968

6 Eckstein H B & MacNab G H Myelomeningocele and hydrocephalus The impact of modern treatment *Lacet I* 842 1966

7 Gitlin D The differentiation and maturation of specific immune mechanisms *Acta Paediat Scand 172* 60 1967

8 Lundstrom R Rubella during pregnancy *Acta Paediat Scand 51* 133 1962

9 Mainland D *Elementary Medical Statistics* W B Saunders London and Philadelphia 1952

10 Mancini G Carbonara A O & Heremans J F Immunochemical quantitation of antigens by single radial immunodiffusion *Immunochemistry 2* 235 1960

11 Manson M M Logan W P D & Loy *Rubella and Other Virus Infections during Pregnancy*—Reports on Public Health and Medical Subjects Her Majesty s Stationary Office 101 Ministry of Health London *1960*

12 Remington J S Millar M J & Brownlee I IgM antibodies in acute toxoplasmosis I Diagnostic significance in congenital cases and a method for their rapid demonstration *Pediatrics 41* 1082 1960

13 Scotti A T & Logan L A specific IgM antibody test in neonatal congenital syphilis *J Pediat 73* 242 1968

14 Sever J & Berendes H W Cord blood gamma M as a screening test for congenital viral infections *The Soc Paed Res* 37th Annual Meeting Pennsylvania 1967

15 Sieber O F Jr Fulginiti V A Brazie J & Umlauf H J Jr In utero infection of the fetus by herpes simplex virus *J Pediat 69* 30 1966

16 Soothill J F Hayes K & Dudgeon J A Immunoglobulins in congenital rubella *Lancet I* 1385 1966

17 St Geme J W Jr & Norev G R Proposed embryopathic relation between mumps virus and primary endocardial fibroelastosis *New Eng J Med 275* 339 1966

18 Stiehm R E Ammann A J & Cherry J D Elevated cord macroglobulins in the diagnosis of intrauterine infections *New Eng J Med 275* 971 1966

19 Stoller A & Collmann R D Incidence of infectious hepatitis followed by Down s syndrome nine months later *Lancet II* 1221 1965

20 Weller T H & Hanshaw J B Virological and clinical observations on cytomegalic inclusion disease *New Eng J Med 266* 1233 1962

21 West C D Hong R & Holland N H Immunoglobulin levels from the newborn period to adulthood and in immunoglobulin deficiency states *J Clinic Invest 41* 2054 1962

22 van Furth R Schmit H M E & Hymans W Immunologic development of the human fetus *J Exp Med 122* 1173 1965

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Table 2 *Urinary testosterone excretion in untreated children with adrenal hyperplasia*

Age	Sex	Testosterone (µg/24 hrs)	Authors	Method
years	M	15 4	Tamm *et al* (1966)	Colorimetric
10 years	M	23 2	Lunn & Diegman (1965)	Fluorimetric
17 years	F	134 0	Vermeulen (1966)	Gas liquid chromatography
13 years	F	216 0	Vermeulen (1966)	Gas liquid chromatography
4 weeks	F	4 8	Camacho & Migeon (1966)	Gas liquid chromatography
6½ years	M	47 3	Camacho & Migeon (1966)	Gas liquid chromatography
14 years	F	86 4	Camacho & Migeon (1966)	Gas liquid chromatography
15 years	F	7 2	Camacho & Migeon (1966)	Gas liquid chromatography

The urinary testosterone and pregnanetriol responses to dexamethasone plus ACTH administration makes an adrenal source for testosterone or its precursor androstenedione highly probable

The testosterone glucuronide measured in this study is formed peripherally and does not necessarily reflect the amount of testosterone to which the cells in the body are exposed It could be that inactive precursors of testosterone circulating in the blood are taken up by the periphery i e the liver and converted there to testosterone and immediately further metabolized to testosterone glucuronide Testosteroneglucuronide from this source does not indicate the amount of testosterone circulating in blood However in untreated children with congenital adrenal hyperplasia high urinary testosterone production rates (3 4 10) and also elevated plasma testosterone and androstenedione levels are found (8 10 13) Androstenedione a not very androgenic steroid in man (5) is in equilibrium in blood with testosterone and Horton & Fraser (8) have suggested that in fact almost all of the increased plasma testosterone in congenital adrenal hyperplasia is the result of increased androstenedione secretion by the adrenals

In the three older patients studied it is difficult to suggest a valid correlation between the level of urinary testosterone excretion and the degree of skeletal advancement In the newborn girls with the seemingly high urinary testosterone excretion no advancement in skeletal maturation was noted, despite the presence of varying degrees of labial fusion Possibly more can be learned from a study of newborn girls who have been exposed in utero to progestational agents as in some of these patients both fusion of labia minora and advancement of skeletal maturation at birth have been noted (1)

SUMMARY

Urinary testosterone 17 ketosteroids and pregnanetriol excretion were determined in seven untreated children with congenital adrenal hyperplasia four of whom were newborn girls with different degrees of fusion of the labia minora

The level of urinary testosterone excretion correlated with the degree of labial fusion in the three newborn girls with the *salt losing* variant, and with the degree of *advancement of* skeletal maturation in the three older children *In the newly born patients the skeletal maturation was not advanced*

In the one patient with the non salt losing variant the urinary testosterone excretion was markedly lower during the thirteenth than the fourth day of life

REFERENCES

1 Breibart S Bongiovanni A M & Eberlein W R Progestins and skeletal maturation *New Eng J Med 268* 255 1963
2 Bush I E *The chromatography of steroids* Pergamon Press Oxford 1961 p 334
3 Camacho A M & Migeon C J Testosterone excretion and production rate in normal adults and in patients with congenital adrenal hyperplasia *J Clin Endocr 26* 893 1966
4 Degenhart, H J Visser H K A, Wilmink R & Frankena L Production and excretion of

Table 1 *Urinary steroid excretion in seven untreated children with congenital adrenal hyperplasia*

Age	Sex	Salt loser	Degree of fusion of labia	Bone age	Testo sterone µg/24 hrs)	11 oxy-17 ks glucu ronide (mg/24 hrs)	11 deoxy-17 ks Glucu ronide (mg/24 hrs)	11 deoxy-17 ks Sulphate (mg/24 hrs)	Pregnan etriol (mg/24 hrs)
5 days	F	Yes	III	0	21	1 2	0 8	3 5	ND
7 days	F	Yes	IV	0	53	0 9	0 8	1 2	0 3
21 days	F	Yes	II	0	12	0 2	0 2	0 6	0 2
4 days					31				
13 days	F	No	IV	0	17				
2½ years	F	No	IV	8 yrs	160	1 7	1 5	2 1	18 0
3 years	M	No	—	12 yrs	176	3 8	8 7	4 5	2 3
3½ years	M	No	—	10 yrs	89	1 6	1 0	1 9	10 6
				(a)	20	0 4			5 1
				(b)	78	1 6			19 1
4 normal children 3 boys and 1 girl 3½ 6 years old				range	1–3	0 7–1 1	0 4–0 6	0 3–0 6	ND

(a) – On 0 25 mg dexamethasone twice daily for 2 months
(b) – On 0 25 mg dexamethasone twice daily for 2 months and 40 I U ACTH Zn for 3 days
ND – Not detected

two patients who were seen at the age of 5 and 7 days In the only newborn girl with the non salt losing variant where the urinary testo sterone excretion could be determined twice during the first weeks of life much less testo sterone was excreted on the 13th than on the 4th day of life

The urinary pregnanetriol excretion in the three newborn girls with the salt losing variant was low (Table 1)

In the three other untreated children all with the non salt losing condition the level of the urinary excretion of testosterone as well as of 17-ketosteroids and pregnanetriol was high (Table 1) There was a marked fall in urinary testosterone and pregnanetriol excre tion after two months of steroid therapy (dexa methasone 0 25 mg twice daily) in one of the three patients tested A rise on the third day of ACTH administration 40 I U ACTH Zn (Organon) whilst the patient was on dexame thasone therapy was also demonstrable (Table 1) In these three older children the advance ment in skeletal maturation (Table 1) per year of life was (8 yr − 2 ¹/ yr) 2 ¹/ yr = 2 2 (12 yr − 3 yr) 3 yr = 3 and (10 yr − 3 ¹/ yr) 3 ¹/ yr = 1 86 and corresponded with the level of urinary testosterone excretion which was 160 µg/d 176 µg/d and 89 µg/d respectively

DISCUSSION

The urinary testosterone excretion corre sponded with the degree of labial fusion in the three newborn girls with the salt losing condi tion In the one patient with the non salt losing variant where the possible influence of age could be studied a marked drop occurred in the urinary testosterone excretion on the 13th day as compared to the 4th day of life In older patients the high excretion of urinary tes tosterone is probably the result of increased stimulation of the adrenal glands (Tables 1 and 2) Although the amount of testosterone excre tion in the urine by the four newborn girls was high compared to older children (Table 1) no definite conclusion can be drawn since to our knowledge urinary testosterone excretion levels have not been reported in healthy newborn children

It is known that in some patients with con genital adrenal hyperplasia the urinary preg nanetriol excretion during the first weeks of life is low and usually after a few months is abnormally high (14)

Table 2 *Urinary testosterone excretion in untreated children with adrenal hyperplasia*

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2 Bush, I E *The chromatography of steroids* Pergamon Press Oxford 1961 p 336
3 Camacho A M & Migeon C J Testosterone excretion and production rate in normal adults and in patients with congenital adrenal hyperplasia *J Clin Endocr* 26 893 1966
4 Degenhart H J Visser H K A Wilmink R & Frankena, L Production and excretion of

testosterone in children with congenital adrenal hyperplasia and precocious puberty *in* A Vermeulen (ed) *Androgens in normal and pathological conditions* Int Congr Series No 101 Excerpta Medica Foundation Amsterdam 1966 p 81
5 Dorfman R I & Shipley R A *Androgen* J Wiley and Sons Inc New York 1956 p 116
6 Epstein E & Zak B A new staining technique for ketosteroid chromatograms *J Clin Endocr 23* 355 1963
7 Greulich W W & Pyle S I *Radiographic atlas of skeletal development of the hand and wrist* Stanford University Press California 1959
8 Horton R & Frasier S D Androstenedione and its conversion to plasma testosterone in congenital adrenal hyperplasia *J Clin Invest 46* 1003 1967
9 Ibayashi H Nakamura M Murakawa S Uchikawa T Tanioka T & Nakao K The determination of urinary testosterone using thin layer chromatography and gas chromatography *Steroids 3* 559 1964
10 Korenman S G Kirschner M A & Lipsett M A Testosterone production in normal and virilised women with the Stein Leventhal syndrome or idiopathic hirsutism *J Clin Endocr 25* 798 1965
11 Lamm N Y & Dingman J I Measurement of testosterone excretion and production rate by glass paper chromatography *J Clin Endocr 25* 563 1965
12 Prader A Der Genitalbefund beim Pseudohermaphroditismus femininus des kongenitalen adrenogenitalen Syndroms *Helv Paediat Acta 9* 231 1954
13 Rivarola M A Saez J M & Migeon C J Studies of androgens in patients with congenital adrenal hyperplasia *J Clin Endocr 27* 624 1967
14 Rudd B T Galal O M & McArthur R G A difficulty in confirming a 21 hydroxylase defect in a neonate *Aust Paediat J 4* 38 1968
15 Tamm J Apostolakis M & Voigt K D The effects of ACTH and HCG on urinary excretion of testosterone in male patients with various endocrine disorders *Acta Endocr* (Kbh) *53* 61 *1966*
16 Vermeulen A I *Androgens in normal and pathological conditions* Int Congr Series No 101 Excerpta Medica Foundation Amsterdam 1966 p 71

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AMINO ACID METABOLISM IN HEREDITARY FRUCTOSEMIA

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Hereditary fructosemia is not a relatively benign condition as previously suggested since lethal cases are reported (11 12 15) The severity of the symptoms will depend upon the age when fructose ingestion is started

In early infancy introduction of fructose into the diet will result in vomiting dystrophy failure to thrive and hepatorenal dysfunction with generalized aminoaciduria (5) The continued ingestion of fructose may result in cirrhosis of the liver ascites edema brain damage convulsions and even death (4 11 12 15) The clinical and biochemical findings are similar to the findings in other metabolic diseases (11)

When fructose is included in the diet at a later age the illness is less severe even asymptomatic Ingestion of fructose results in vomiting or hypoglycemic attacks Most patients seem to develop a strong aversion to sweets and fruits and thereby protect themselves against the noxious factor (4 5)

Three cases with symptoms in early infancy one with a lethal course will be presented in this paper The amino acid metabolism in the acute stage of this disease has not previously been studied in detail and it is the main purpose of the paper to report such studies with special reference to similar studies on patients with acute tyrosinosis

METHODS

The plasma and urinary values were obtained by the standard micromethods used in our laboratory

The true blood glucose level was determined by the o-toluidin method (9) and the total blood sugar by that of Hagedorn & Jensen (7) A 20 g/100 ml fructose solution was used in the intravenous fructose tolerance test.

Quantitative determination of serum phenylalanine and tyrosine was performed according to Wong et al (19) Amino acids were determined in serum ascitic fluid and urine on a Technicon Amino Acid Autoanalyzer as previously described (16) The phenolic acids were determined semiquantitatively by paper chromatography (1)

The F 1 P aldolase[1] and the F 1 6-DP aldolase activities in liver homogenates were assayed according to the methods of Wolf (18) and Bruns & Bergmeyer (3)

CASE REPORTS

Case 1 I J H F a 2770 g boy was born June 12 1968 after a normal twin pregnancy The parents were unrelated older siblings were normal and there was no history of metabolic disturbances in the family His dizygous twin was healthy and weighed 3200 g

He was breast fed the first week and then placed on a milk formula containing sucrose Vomiting and loss of weight ensued almost immediately At age 2 weeks he was hospitalized and treated with frequent meals and methylatropin without improvement

On admission to this hospital at the age of 4 weeks he was dystrophic icteric and 300 g below his birth weight His abdomen was distended a firm liver was

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[1] ATP D-fructose 1 phosphotransferase (EC 2.7 1 3)
Fructose 1 6-diphosphate D-glyceraldehyde 3 phosphate-lyase (EC 4 1.2 b)

Table 1 *Main laboratory findings in three cases of acute hereditary fructosemia*

Patient	Case 1 (J H F) Weeks				Case 2 (E G) Weeks				Case 3 (B R) Months		
Age	4	6	6½	p m	4	7/8	21	22	4	5½	6
Blood											
Calcium (mEq/l)	4 6	5 5	3 7	3 1		5 2	5 3		4 3	4 3	
Phosphorus (mg/100 ml)	6 7			7 4		6 6	6 2		2 9	3 2	
Serum protein (g/100 ml)	5 9		4 6	4 0	5 0		6 4	4 9		3 9	5[illegible]
PP index (per cent)	29	14	45	42	5	90	5		44	[illegible]4	15
Alkaline phosphatase (IU)	125	73		34	175	130	228		135	140	
SGOT (IU)	96	198		135	260	28	500	230	100	88	
SGPT (IU)	93	154		75	340	29		610	65		
Bilirubin (mg/100 ml)	5 0			5 5	11 0	0 9	0 1		1 8		
Blood glucose (mg/100 ml)	42	20	40	10	45		31		74		
Bicarbonate (mEq/l)	26	20	22		13	23	15	17	16	21	18
Urinary reactions											
Protein	–	–	+	+	–	–	+		–	+ +	+
Benedict	–	+	+	+			+	+	+	+	+
Clinistix	–	–	+	+	–		+		+	+	+
2–4 Dinitrophenylhydrazine	–	–	(+)	–			+ +	(+)			

p m = post mortem

palpable 2 cm below the costal margin The only abnormal laboratory findings consisted of a low prothrombin/proconvertin index and slightly elevated serum bilirubin and transaminases Initially reducing substances could not be demonstrated in the urine (Table 1) Galactosemia was the suspected diagnosis but in spite of a lactose free diet the vomiting continued At the age of 6 weeks a reducing substance in the urine was identified as fructose

During this time the patient had recurrent apnoeic episodes requiring artificial ventilation In addition the patient developed ascites generalized edema hypoelectrolytemia and hypoglycemia to 14 mg/100 ml at one point The therapy included human milk feeding and infusions of glucose and bicarbonate but this was to no avail During an apnoeic episode at 7 weeks of age the patient developed generalized convulsions and died

At necropsy the liver was moderately enlarged greenish brown with a finely granular surface and firm consistency Microscopically extensive periportal fibrosis as well as some centrilobular fibrosis was noted In addition the liver showed marked proliferation of biliary ducts with bile pigment included in the liver cells often forming pseudoacini

The kidneys appeared normal macroscopically but showed the proximal convoluted tubules to be slightly distended and to contain eosinophilic material upon microscopy Deposits of amorphous material which stained positive for calcium were found in and around the cortical tubules and were associated with some degeneration of the epithelial cells

Macroscopically the brain appeared normal though small with a whitish surface and gleatinous consistency in cut sections Microscopy showed a pronounced shrinkage of nerve cells with wide pericellular spaces in the cortex the striatum and basal ganglia No apparent reduction in the number of nerve cells had taken place Some glial nuclei were slightly enlarged but not to the extent described in chronic liver diseases The white matter of the hemispheres had a loose structure with stellate glial cells containing sudanophilic lipid material in their cytoplasm A few sudanophilic macrophages were seen around the vessels Luxol fast blue staining showed myelin to be present in the internal capsule and lower brain stem but absent in the cerebral hemispheres

Case 2 E G a 3500 g girl was born Sept 8 1968 at term after an uneventful pregnancy The parents were unrelated and two siblings were normal

The child was breast fed for the first few days and was then placed on a milk formula containing sucrose At 2 / weeks she became weak lost her appetite and vomited after each meal She was admitted to a local hospital being dystrophic and weighing 500 g below her birth weight At this time a bulging fontanel was noted

On admission to this hospital at the age of one month an additional finding was that of a palpable firm liver A spinal puncture showed a slightly increased pressure and an EEG showed asymmetry and reduced activity over the left temporal region Following admission the child became weaker icteric showed an increased bleeding tendency and developed severe metabolic acidosis The transaminases were markedly increased (Table 1) The proteinuria mellituria and hematuria indicated renal dysfunction A generalized aminoaciduria with a high excretion of tyrosine and phenolic acids and an elevated serum tyrosine pointed to an acute tyrosinosis A phenylalanine tyrosine restricted diet containing small amounts of sucrose resulted in a marked clinical im

provement with a trend to normalization of liver function, aminoacidemia and aminoaciduria. The child was discharged doing well on a diet containing 80 mg of both phenylalanine and tyrosine per kg per day and 9 g sucrose per day.

She did well at home and her weight increased. At 19 weeks of age she was readmitted for control. The liver was firm and palpable and although the fontanel was still bulging, the head circumference was within normal limits. Both serum phenylalanine and tyrosine were normal and a transitory type of tyrosinosis was considered. A phenylalanine loading test was performed and interpreted as normal. At 21 weeks of age she was placed on a normal diet. One day later the serum phenylalanine and tyrosine levels were slightly increased but the normal diet was continued. She then developed ascites and started to vomit, and a severe metabolic acidosis occurred which was treated with bicarbonate. Besides markedly increased values of serum phenylalanine and tyrosine, generalized aminoaciduria, mellituria and high excretion of phenolic acids were noted. Again the phenylalanine-tyrosine restricted diet was started and she improved. The normal diet contained twice as much phenylalanine and tyrosine as the restricted diet, and the sucrose content was 38 g per day compared with the 9 g of the restricted diet. 38 g of sucrose was added to the restricted diet and this produced immediate vomiting and mellituria. The blood sugar was found to be 32 mg/100 ml on this sucrose intake. In another period when 38 g lactose was added to the restricted diet no reaction was observed.

A fructose tolerance test was performed (Fig. 1) during which the patient collapsed and glucose had to be given in large amounts. True blood glucose was 15 mg/100 ml confirming the diagnosis of fructosemia.

Phenylalanine and tyrosine were now added to the diet without any deleterious effects, and the child was placed on a fructose free diet followed by normalization of the serum and urine values.

Case 3. B. R., a 3600 g girl, was born Febr. 6, 1963 after an uneventful pregnancy. The parents were unrelated and two siblings were normal.

The child was breast fed for the first 14 days, and was then placed on a milk formula containing sucrose. She was icteric for some days when three weeks old. At 6 weeks she became feverish and started to vomit shortly after each meal. The tendency to vomit continued and at 4 months she was hospitalized. Dystrophia, jaundice and an enlarged liver were noted. The only abnormal laboratory findings consisted of a low prothrombin/proconvertin index and increased serum bilirubin and transaminases. In the urine reducing substance and protein were found.

On admission to this hospital some days later she was extremely dystrophic, her abdomen was distended with ascites and a firm liver was palpable 3 cm below the costal margin. Increased serum transaminases, a metabolic acidosis and a low prothrombin/proconvertin index were present (Table I). Because of an

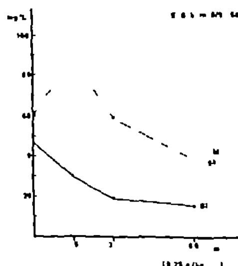


Fig. 1. Intravenous fructose tolerance test in case 2 injected 0.25 g fructose per kg body weight.

intercurrent fever, sepsis was considered. Blood cultures gave, however, no evidence for this diagnosis. As galactose was found in the urine, she was placed on a lactose free diet, but the red cell galactose-1-phosphate uridyl transferase level turned out to be normal. The child got worse and repeated abdominal paracenteses were performed, followed by plasma transfusions. In addition a generalized aminoaciduria with a particularly high excretion of tyrosine was noted.

In her 7th and 8th months the child clinically improved although hepatomegaly and tyrosyluria were present. The serum amino acids were normal and she was discharged without any definite diagnosis. On reexamination at 10 months she was clinically quite normal. All the laboratory data and the amino acids in both serum and urine were within normal limits. The diagnosis of acute tyrosinosis was suggested when the previous data was reexamined but the treatment course was not explainable.

On control 6 years after the last admittance she was mentally and physically normal. The parents said spontaneously that the girl had developed a strong aversion against all sorts of sweets and fruits. Reexamination of her food intake during the first year of life revealed that she had developed her first symptoms after the milk formula containing sucrose had been given. The clinical improvement came when she was changed from the milk formula to whole milk. Thereafter she protected herself by developing the aversion against sweets.

The anamnestic and clinical data indicated the diagnosis of fructosemia, although liver enzyme studies and fructose tolerance test had not been performed.

UTP: α-D-galactose-1-phosphate uridyltransferase (EC 2.7.7.10).

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Serum protein (g/100 ml)	5 9		4 6	4 0	5 0		6 4	4 9		3 9	5 8
PP index (per cent)	29	14	45	42	5	90	5		44	24	15
Alkaline phosphatase (IU)	125	73		34	175	130	228		135	140	
SGOT (IU)	96	198		135	260	28	500	230	100	83	
SGPT (IU)	93	154		75	340	29		610	65		
Bilirubin (mg/100 ml)	5 0			5 5	11 0	0 9	0 1		1 8		
Blood glucose (mg/100 ml)	42	20	40	10	45		31		74		
Bicarbonate (mEq/l)	26	20	22		13	23	15	17	16	21	18
Urinary reactions											
Protein	−	−	+	+	−	−	+		−	++	+
Benedict	−	+	+	+			+	+	+	+	+
Clinistix	−	−	+	+	−		+		+	+	+
2–4 Dinitrophenylhydrazine	−	−	(+)	−			++	(+)			

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provement with a trend to normalization of liver function ammonaemia and aminoaciduria. The child was discharged doing well on a diet containing 80 mg of both phenylalanine and tyrosine per kg per day and 9 g sucrose per day.

She did well at home and her weight increased. At 19 weeks of age she was readmitted for control. The liver was firm and palpable and although the fontanel was still bulging the head circumference was within normal limits. Both serum phenylalanine and tyrosine were normal and a transitory type of tyrosinosis was considered. A phenylalanine loading test was performed and interpreted as normal. At 21 weeks of age she was placed on a normal diet. One day later the serum phenylalanine and tyrosine levels were slightly increased but the normal diet was continued. She then developed ascites and started to vomit, and a severe metabolic acidosis occurred which was treated with bicarbonate. Besides markedly increased values of serum phenylalanine and tyrosine generalized aminoaciduria, mellituria and high excretion of phenolic acids were noted. Again the phenylalanine tyrosine restricted diet was started and she improved. The normal diet contained twice as much phenylalanine and tyrosine as the restricted diet and the sucrose content was 38 g per day compared with the 9 g of the restricted diet. 38 g of sucrose was added to the restricted diet and this produced immediate vomiting and mellituria. The blood sugar was found to be 32 mg/100 ml on this sucrose intake. In another period when 38 g lactose was added to the restricted diet no reaction was observed.

A fructose tolerance test was performed (Fig. 1) during which the patient collapsed and glucose had to be given in large amounts. True blood glucose was 13 mg/100 ml confirming the diagnosis of fructosemia.

Phenylalanine and tyrosine were now added to the diet without any deleterious effects and the child was placed on a fructose free diet followed by normalization of the serum and urine values.

Case 3. B. R. a 3600 g girl was born Febr. 6 1963 after an uneventful pregnancy. The parents were unrelated and two siblings were normal.

The child was breast fed for the first 14 days and was then placed on a milk formula containing sucrose. She was icteric for some days when three weeks old. At 6 weeks she became feverish and started to vomit shortly after each meal. The tendency to vomit continued and at 4 months she was hospitalized. Dystrophia, jaundice and an enlarged liver were noted. The only abnormal laboratory findings consisted of a low prothrombin/proconvertin index and increased serum bilirubin and transaminases. In the urine reducing substances and protein were found.

On admission to this hospital some days later she was extremely dystrophic, her abdomen was distended with ascites and a firm liver was palpable 3 cm below the costal margin. Increased serum transaminases, a metabolic acidosis and a low prothrombin/proconvertin index were present (Table 1). Because of an

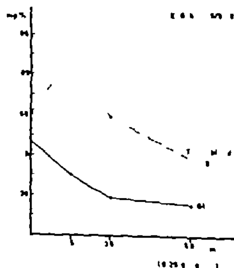


Fig. 1. Intravenous fructose tolerance test in case 2 injected 0.25 g fructose per kg body weight.

tercurrent fever sepsis was considered. Blood cultures gave however no evidence for this diagnosis. As galactose was found in the urine she was placed on a lactose free diet but the red cell galactose 1 phosphate uridyl transferase level turned out to be normal. The child got worse and repeated abdominal paracenteses were performed followed by plasma transfusions. In addition a generalized aminoaciduria with a particularly high excretion of tyrosine was noted.

In her 7th and 8th months the child clinically improved although hepatomegaly and tyrosyluria were present. The serum amino acids were normal and she was discharged without any definite diagnosis. On reexamination at 10 months she was clinically quite normal. All the laboratory data and the amino acids in both serum and urine were within normal limits. The diagnosis of "acute tyrosinosis" was suggested when the previous data was reexamined but the transient course was not explainable.

On control 6 years after the last admittance she was mentally and physically normal. The parents said spontaneously that the girl had developed a strong aversion against all sorts of sweets and fruits. Reexamination of her food intake during the first year of life revealed that she had developed her first symptoms after the milk formula containing sucrose had been given. The clinical improvement came when she was changed from the milk formula to whole milk. Thereafter she protected herself by developing the aversion against sweets.

The anamnestic and clinical data indicated the diagnosis of fructosemia although liver enzyme studies and fructose tolerance t s ad not been performed.

UTP = D-galactose 1 phosphate uridyltransferase (EC 2.7.7.10)

Table 1 *Main laboratory findings in three cases of acute hereditary fructosemia*

Patient	Case 1 (J H F) Weeks				Case 2 (E O) Weeks				Case 3 (B R) Months		
Age	4	6	6½	p m	4	7/8	21	22	4	5½	6
Blood											
Calcium (mEq/l)	4 6	5 5	3 7	3 1		5 2	5 3		4 3	4 3	
Phosphorus (mg/100 ml)	6 7			7 4		6 6	6 2		2 9	3 [illegible]	
Serum protein (g/100 ml)	5 9		4 6	4 0	5 0		6 4	4 9		3 9	5 8
PP index (per cent)	29	14	45	42	5	90	5		44	24	15
Alkaline phosphatase (IU)	125	73		34	175	130	228		135	140	
SGOT (IU)	96	198		135	260	28	500	230	100	88	
SGPT (IU)	93	154		75	340	29		610	65		
Bilirubin (mg/100 ml)	5 0			5 5	11 0	0 9	0 1		1 8		
Blood glucose (mg/100 ml)	42	20	40	10	45		31		74		
Bicarbonate (mEq/l)	26	20	22		13	23	15	17	16	21	18
Urinary reactions											
Protein	–	–	+	+	–	–	+		–	++	+
Benedict	–	+	+	+			+	+	+	+	+
Clinistix	–	–	+	+	–		+		+	+	+
2–4 Dinitrophenylhydrazine	–	–	(+)	–			++	(+)			

p m = post mortem

palpable 2 cm below the costal margin The only abnormal laboratory findings consisted of a low prothrombin/proconvertin index and slightly elevated serum bilirubin and transaminases Initially reducing substances could not be demonstrated in the urine (Table 1) Galactosemia was the suspected diagnosis but in spite of a lactose free diet the vomiting continued At the age of 6 weeks a reducing substance in the urine was identified as fructose

During this time the patient had recurrent apnoeic episodes requiring artificial ventilation In addition the patient developed ascites generalized edema hypoelectrolytemia and hypoglycemia to 14 mg/100 ml at one point The therapy included human milk feeding and infusions of glucose and bicarbonate but this was to no avail During an apnoeic episode at 7 weeks of age the patient developed generalized convulsions and died

At necropsy the liver was moderately enlarged greenish brown with a finely granular surface and firm consistency Microscopically extensive periportal fibrosis as well as some centrilobular fibrosis was noted In addition the liver showed marked proliferation of biliary ducts with bile pigment included in the liver cells often forming pseudoacini

The kidneys appeared normal macroscopically but showed the proximal convoluted tubules to be slightly distended and to contain eosinophilic material upon microscopy Deposits of amorphous material which stained positive for calcium were found in and around the cortical tubules and were associated with some degeneration of the epithelial cells

Macroscopically the brain appeared normal though small with a whitish surface and gleatinous consistency in cut sections Microscopy showed a pronounced shrinkage of nerve cells with wide pericellular spaces in the cortex the striatum and basal ganglia No apparent reduction in the number of nerve cells had taken place Some glial nuclei were slightly enlarged but not to the extent described in chronic liver diseases The white matter of the hemispheres had a loose structure with stellate glial cells containing sudanophilic lipid material in their cytoplasm A few sudanophilic macrophages were seen around the vessels Luxol fast blue staining showed myelin to be present in the internal capsule and lower brain stem but absent in the cerebral hemispheres

Case 2 E G a 3500 g girl was born Sept 8 1968 at term after an uneventful pregnancy The parents were unrelated and two siblings were normal

The child was breast fed for the first few days and was then placed on a milk formula containing sucrose At 2 / weeks she became weak lost her appetite and vomited after each meal She was admitted to a local hospital being dystrophic and weighing 500 g below her birth weight At this time a bulging fontanel was noted

On admission to this hospital at the age of one month an additional finding was that of a palpable firm liver A spinal puncture showed a slightly increased pressure and an EEG showed asymmetry and reduced activity over the left temporal region Following admission the child became weaker icteric showed an increased bleeding tendency and developed severe metabolic acidosis The transaminases were markedly increased (Table 1) The proteinuria mellituria and hematuria indicated renal dysfunction A generalized ammoaciduria with a high excretion of tyrosine and phenolic acids and an elevated serum tyrosine pointed to an acute tyrosinosis A phenylalanine tyrosine restricted diet containing small amounts of sucrose resulted in a marked clinical im

Table 4 *Excretion of the urinary amino acids and the phenolic acids in three cases of acute hereditary fructosemia expressed as μmoles/mg creatinine*

	Normal[a] 11 wks	Case 1 6½ wks	Case 2 4 wks	Case 3 4 months
osphoethanolamine	0 02–0 33	1 14	0 50	0 82
aurine	0 63–3 43	1 58	2.0	0 36
ethionine sulfoxide	0	0.86	0 40	0 35
ydroxyproline	0 56–0 93	11 30	6 00	5 80
spartic acid	0–0 10		6 58	5 18
hreonine	0 54–0 58	46 37	26 00	36 96
erine	1 10–1 64	Large	14 50	37 65
lutamic acid	0 04–0.21	1 72	4 50	2 10
roline	0 01–0 21	33 63	37 20	10 32
itrulline	0	3 82	6 60	4 95
lycine	2.60–2.79	Large	75 90	40 79
lanine	1 06–1 37	36 63	72 90	27 49
-amino-n-butyric acid	0–0 24	0 47	0 80	0 79
aline	0 02–0 27	5 67	9 90	2 70
Cystine	0 22	2 50	2 00	1 30
Methionine	Tr –0 8	5 45	4 80	1 58
Cystathionine	0	1 57	2 70	0 69
soleucine	Tr –0 16	0 63	1 20	0 22
Leucine	Tr –0 20	2.31	3.20	1 57
Tyrosine	0 24–0 37	16 10	25 50	10 92
Phenylalanine	0–0 16	10 52	6 60	4 54
β aminoisobutyric acid	0 89–2 94	2 96	0 76	0 66
Ethanolamine	0 44–0 62	5 71	5 40	2 60
Hydroxylysine	0	0 23	0 10	0 15
2 4-diaminobutyric acid }		2 56	2 20	1 14
Ornithine }	Tr –0 12			
Lysine }	0 36–0 58	19 55	11 70	9 96
1 methylhistidine }	0 08			
Histidine }	1 28–1 82	41 22	22 70	18 34
3 methylhistidine }	0 14–0 18			
Carnosine	0 38–0 40	0 16	0 36	Tr
Arginine	0 07–0 10	0 16	0 03	0 15
p-hydroxy phenyl lactic acid		33 5	33 5	4 4
p-hydroxy phenyl acetic acid		3 9	23 0	2,6
Tyrosine		16 1	25 5	10 9

[a] Armstrong *et al* (2)

In case 2 the excretion of tyrosine was about 70 times the normal phenylalanine about 40 times and proline about 180 times The tyrosine and phenylalanine excretion in case 3 was each about 30 times the normal and proline about 50 times

The excretion of the phenolic acids *p*-hydroxy phenyl lactic acid and *p*-hydroxy phenyl acetic acid was markedly elevated in cases 1 and 2 and slightly elevated in case 3 (Table 4)

A phenylalanine tolerance test was performed in case 2 when she was ingestion a small amount of sucrose The serum phenylalanine increased to a maximum of 8 1 mg/100 ml after 30 min and the serum tyrosine to a maximum of 2 6 mg/100 ml The findings were interpreted as normal

DISCUSSION

The diagnosis of fructosemia was suggested in case 1 when the patient collapsed was hypoglycemic and had fructosuria after ingestion of fructose Cases 2 and 3 were diagnosed on the basis of clinical and laboratory evidence and the improvement on a therapeutic trial

These cases of fructosemia in early infancy illustrate that the onset and severity of the symptoms are determined by the age at which fructose is introduced into the diet The symptoms do not necessarily occur immediately but

Table 2 *Enzyme activity in liver homogenate in a case of acute hereditary fructosemia expressed as μmoles/g/hour*

	F 1 P aldolase	F 1 6-DP aldolase	F 1 6-DP aldolase/ F 1 P aldolase
Case 1 (J H F)	43 6	394 4	9 25
Normal liver	623 6	782 7	1 25

LABORATORY INVESTIGATIONS

All three cases had normal serum electrolytes determined on several occasions, except for the periods of collapse after fructose ingestion They had low plasma protein levels and developed metabolic acidosis following prolonged ingestion of fructose

Icterus was observed in cases 1 and 2 and was reported in case 3 They all had a slightly elevated alkaline phosphatase and a low prothrombin/proconvertin index which increased after vitamin K injections The transaminases SGOT and SGPT were elevated in all three cases and were particulary high during an acute episode in case 2 All values were normalized on a fructose free diet

In cases 1 and 2, serum phosphorus was not determined immediately after fructose ingestion and was found to be within normal limits or in the upper range of normal In case 3 however the serum phosphorus levels were low increasing as the patient improved

The blood sugar was found markedly decreased immediately after fructose ingestion in cases 1 and 2 Severe hypoglycemia occurred following intravenous fructose loading in case 2

Liver enzymes Enzymatic activity in liver was assayed in case 1 only (Table 2) The liver was removed immediately after death and kept frozen (- 20°C) until investigated The ratio F 1 6 DP aldolase/F 1 P aldolase which normally should be 1 0 was found to be 9 25 in our case To exclude a reduction of the F-1 P aldolase secondary to a generalized liver disease, the β glucuronidase[1] was determined (17) and found to be within normal limits

[1] β D-glucuronide glucuronohydrolase (EC 3 2 1 31)

Serum and urinary amino acids In all three cases generalized aminoacidemia (Table 3) and generalized aminoaciduria (Table 4) were found In case 3 only ascitic fluid and not se rum was investigated but it is assumed that ascitic fluid is equilibrated with serum For the results of the individual amino acids see Tables 3 and 4

In case 1 the serum tyrosine was about 3 times the normal and methionine about 16 times The serum tyrosine in case 2 was about 1 1/2 times the normal and methionine about 2 times In case 3 the amino acids in the ascitic fluid were within normal limits except for methionine which was about 6 times the normal

The urinary excretion of tyrosine in case 1 was about 40 times the normal, phenylalanine about 65 times and proline about 160 times

Table 3 *Serum amino acids in two cases and ascitic fluid amino acids in one case of acute hereditary fructosemia expressed as μmol/liter*

	Normal[a] 12 wks	Case 1 6½ wks	Case 2 4 wks	Case 3[b] 4 months
Taurine	74–216	401	197	40
Methionine sulfoxide		25	7	17
Hydroxyproline		150	70	
Aspartic acid	Tr – 17	18	77	
Threonine	114–335	615	233	65
Serine	94–243	888	463	143
Glutamic acid	20–107	135	309	31
Proline	107–277	420	387	198
Citrulline	9– 29	110	68	41
Glycine	224–514	2468	806	97
Alanine	236–410	865	788	212
α amino n butyric acid	6– 29	46	19	10
Valine	80–246	192	177	73
Cystine	35– 84	124	26	28
Methionine	9– 41	664	70	239
Cystathionine		28	26	3
Isoleucine	27– 53	67	53	26
Leucine	47–109	134	121	42
Tyrosine	42– 99	316	113	84
Phenylalanine	42–110	177	91	65
Ethanolamine	26– 92	282	40	16
Ornithine	49–151	288	131	34
Lysine	114–269	576	292	102
Histidine	49–114	166	228	39
Arginine	27– 88	168	57	51

[a] Perry *et al* (14)
[b] Ascitic fluid

ance test has been performed which gave a normal rise in both blood glucose and fructose

The findings presented in this paper support the view that the clinical and biochemical picture of "acute tyrosinosis" may arise from different metabolic disorders. Furthermore they support the concept that the hepatorenal dysfunction observed in tyrosinosis or hereditary tyrosinemia is secondary to some other metabolic defect than one in the tyrosine pathway

SUMMARY

Three patients with hereditary fructosemia are presented and discussed one with a lethal course. In the acute form of fructosemia after prolonged administration of fructose infants may show similar clinical laboratory and histological findings as infants with "acute tyrosinosis". Both disorders exhibit hepatomegaly, edema, ascites, generalized aminoaciduria and striking excretion of phenolic acids. These findings support the view that the clinical and biochemical picture of "acute tyrosinosis" may arise from different metabolic disorders. Furthermore they support the concept that the hepatorenal dysfunction observed in tyrosinosis or hereditary tyrosinemia is secondary to some other metabolic defect than one in the tyrosine pathway

REFERENCES

1 Armstrong M D, Shaw K N & Wall P E. The phenolic acids of human urine: paper chromatography of phenolic acid. *J Biol Chem* 218 793 1956

2 Armstrong M D, Yates K N & Connelly J P. Amino acid excretion of newborn infants during the first twenty four hours of life. *Pediatrics* 33 975 1964

3 Bruns F H & Bergmeyer H U. Fructose 1 6-diphosphate aldolase. In H U Bergmeyer (ed.) *Methods of enzymatic analysis*. Academic Press New York 1965 p 724

4 Froesch E R, Prader A, Labhart, A, Stuber H W & Wolf H P. Die hereditäre Fructoseintoleranz, eine bisher nicht bekannte Stoffwechselstörung. *Schweiz Med Wschr* 87 1168 1957

5 Froesch E R, Wolf H P, Baitsch H, Prader A & Labhart A. Hereditary fructose intolerance. *Amer J Med* 34 151 1963

6 Gentz, J, Jagenburg, R & Zetterstrom R. Tyrosinemia. *J Pediat* 66 670 1965

7 Hagedorn, H C, Halstrom F & Jensen N. Hurtige metoder til bestemmelse av blodsukker ved kaliumferricyanid. *Hospitalstid* 78 1193 1935

8 Halvorsen S, Pande H, Loken Aa C & Gjessing L R. Tyrosinosis. *Arch Dis Child* 41 238 1966

9 Haltmann E. Rapid specific method for determination of aldosaccharides in body fluids. *Nature* 183 108 1959

10 Lelong M, Alagille D, Gentil C, Colin J, Mercie C, Tobin, J & Bouquier J. L'intolérance héréditaire au fructose. *Arch Franc Pediat* 19 841 1962

11 Levin B, Snodgrass G J, Oberholzer V G, Burgess E A & Dobbs R H. Fructosaemia. *Amer J Med* 45 826 1968

12 Lindemann R, Gjessing L R, Merton B & Halvorsen S. Fructosemia/"Acute tyrosinosis". *Lancet* I 891 1969

13 Odièvre M, Gentier M & Rieu D. Intolérance héréditaire au fructose du nourrisson. *Arch Franc Pediat* 26 433 1969

14 Perry T L, Hardwick D F, Dixon G H, Dolman C L & Hansen S. Hypermethioninemia. A metabolic disorder associated with cirrhosis, islet cell hyperplasia and renal tubular degeneration. *Pediatrics* 36 236 1965

15 Sacrez, R, Juif J, Metais P, Sofatzis J & Dourof N. Un cas mortel d'intolérance héréditaire au fructose. *Pediatrie* 17 875 1962

16 Seip M, Stern Johnsen J, Vellan J E & Gjessing L R. Dietary treatment of cystinosis. *Acta Pediat Scand* 57 409 1968

17 Winsnes A. Studies of the activation in vitro of glucuronyltransferase. *Biochim Biophys Acta* 191 297 1969

18 Wolf H P. 1 Phosphofructoaldolase. In H U Bergmeyer (ed.): *Methods of enzymatic analysis*. Academic Press New York 1965 p 732

19 Wong P W K, O'Flynn M O & Inouye T. Micromethods for measuring phenylalanine and tyrosine in serum. *Clin Chem* 10 1098 1964

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may develop gradually one or more weeks after the regular ingestion of fructose

The acute form of fructosemia in early infancy may show the same clinical and biochemical picture as acute tyrosinosis (8, 12) Both conditions may cause hepatomegaly ascites, edema pathological liver function tests, proteinuria, mellituria and SGOT and SGPT elevation In our cases these were more marked in fructosemia than in tyrosinosis

In fructosemia, the serum phosphorus usually decreases after ingestion of fructose In our cases phosphorus was not determined immediately after fructose ingestion and was found at the upper limit of normal in cases 1 and 2 and moderately low in case 3 Even though considerable phosphaturia may occur (11) rickets have not been described in fructosemia although this is a constant finding in tyrosinosis (6 8) The reason may be that patients with undiagnosed fructosemia die before rickets develops or that renal tubular function is normalized in those who develop an aversion to sweets

The serum amino acids are elevated both in fructosemia and acute tyrosinosis but in fructosemia aminoacidemia appears to be more generalized The methionine level is very high in both disorders in the acute stage and tyrosine is elevated paticulary in acute tyrosinosis A marked phenolic acid excretion and generalized aminoaciduria are found in both disorders with proline excretion being particulary high in fructosemia and only moderately increased in acute tyrosinosis In the cases with fructosemia the tyrosyluria disappeared as fructose was removed from the diet

A study of the liver in case 1 showed cirrhosis stenosis of the hepatic duct and probable obliteration of the cystic duct The stenosis may account for some of the hepatic fibrosis and biliary proliferation but the picture is similar to that described previously in fructosemia (10 13 15) Degeneration of the renal tubular epithelium has been described in fructosemia while associated calcified deposits have not been reported

The findings of retarded myelination and lipid storage in the glial cells of the brain seem to indicate that the metabolic derangement may be interfering with myelination a very active process during this period of development

Comparing the histological findings with those described in tyrosinosis (8) there are also striking similarities Signs of regenerating liver parenchyma which are pronounced in tyrosinosis were lacking in the present case It should however be remembered that case 1 was only 7 weeks old, while cases of tyrosinosis previously examined have all died at later ages

Mental changes in tyrosinosis have been observed clinically (6) Sudanophilic granules in glial cells were observed in one out of the three cases of tyrosinosis (8) The picture of enlarged naked glial nuclei or liver glia in cortex and striatum that dominated the picture in those three cases, was not prominent in the present case Such changes are however usually combined with more prolonged cases of liver diseases Considering individual differences and age differences the findings described in tyrosinosis and fructosemia both in liver kidneys and brain are closely related

In fructosemia the marked derangement in tyrosine metabolism occuring after fructose ingestion judging from the excreted metabolites is probably due to the decreased activity of p hydroxyphenylpyruvate hydroxylase[1] The activity of this enzyme is most easily influenced during infancy There is also a derangement in methionine metabolism, as in acute tyrosinosis The proline excretion should be investigated in future cases of fructosemia

Based on our present experience it is not possible to make a differential diagnosis between fructosemia and acute tyrosinosis on the basis of the urine amino acid excretion serum amino acids and the phenolic acid excretion when the child is on a normal diet A fructose tolerance test must be performed to make a definite diagnosis In two of our chronic cases of tyrosinosis a fructose tolera

[1] (EC 1 99 1 14)

-ine test has been performed which gave a normal rise in both blood glucose and fructose

The findings presented in this paper support the view that the clinical and biochemical picture of "acute tyrosinosis" may arise from different metabolic disorders. Furthermore they support the concept that the hepatorenal dysfunction observed in tyrosinosis or hereditary tyrosinemia is secondary to some other metabolic defect than one in the tyrosine pathway.

SUMMARY

Three patients with hereditary fructosemia are presented and discussed, one with a lethal course. In the acute form of fructosemia after prolonged administration of fructose infants may show similar clinical, laboratory and histological findings as infants with "acute tyrosinosis". Both disorders exhibit hepatomegaly, edema, ascites, generalized aminoaciduria and striking excretion of phenolic acids. These findings support the view that the clinical and biochemical picture of "acute tyrosinosis" may arise from different metabolic disorders. Furthermore they support the concept that the hepatorenal dysfunction observed in tyrosinosis or hereditary tyrosinemia is secondary to some other metabolic defect than one in the tyrosine pathway.

REFERENCES

1 Armstrong, M. D., Shaw, K. N. & Wall, P. E.: The phenolic acids of human urine: paper chromatography of phenolic acid. *J Biol Chem 218*: 293, 1956.

2 Armstrong, M. D., Yates, K. N. & Connelly, J. P.: Amino acid excretion of newborn infants during the first twenty-four hours of life. *Pediatrics 33*: 975, 1964.

3 Bruns, F. H. & Bergmeyer, H. U.: Fructose-1,6-diphosphate aldolase. H. U. Bergmeyer (ed.): *Methods of enzymatic analysis*. Academic Press, New York 1965, p. 724.

4 Froesch, E. R., Prader, A., Labhart, A., Stuber, H. W. & Wolf, H. P.: Die hereditäre Fructoseintoleranz, eine bisher nicht bekannte Stoffwechselstörung. *Schweiz Med Wschr 87*: 1168, 1957.

5 Froesch, E. R., Wolf, H. P., Baitsch, H., Prader, A. & Labhart, A.: Hereditary fructose intolerance. *Amer J Med 34*: 151, 1963.

6 Gentz, J., Jagenburg, R. & Zetterström, R.: Tyrosinemia. *J Pediat 66*: 670, 1965.

7 Hagedorn, H. C., Halström, F. & Jensen, N.: Hurtige metoder til bestemmelse av blodsukker ved kaliumferricyanid. *Hospitalstid 78*: 1193, 1935.

8 Halvorsen, S., Pande, H., Løken, A. C. & Gjessing, L. R.: Tyrosinosis. *Arch Dis Child 41*: 238, 1966.

9 Holtman, E.: Rapid specific method for determination of aldosaccharides in body fluids. *Nature 183*: 108, 1959.

10 Lelong, M., Alagille, D., Gentil, C., Colin, J., Mercier, C., Tubin, J. & Bousquet, J.: L'intolérance héréditaire au fructose. *Arch Franc Pediat 19*: 841, 1962.

11 Levin, B., Snodgrass, G. J., Oberholzer, V. G., Burgess, E. A. & Dobbs, R. H.: Fructosaemia. *Amer J Med 45*: 826, 1968.

12 Lindemann, R., Gjessing, L. R., Merton, B. & Halvorsen, S.: Fructosemia / "Acute tyrosinosis". *Lancet I*: 891, 1969.

13 Odievre, M., Gautier, M. & Rieu, D.: Intolérance héréditaire au fructose du nourrisson. *Arch Franc Pediat 26*: 433, 1969.

14 Perry, T. L., Hardwick, D. F., Dixon, G. H., Dolman, C. L. & Hansen, S.: Hypermethioninemia. A metabolic disorder associated with cirrhosis, islet cell hyperplasia, and renal tubular degeneration. *Pediatrics 36*: 236, 1965.

15 Sacrez, R., Juif, J., Metais, P., Sofatzis, J. & Doutot, N.: Un cas mortel d'intolérance héréditaire au fructose. *Pédiatrie 17*: 875, 1962.

16 Seip, M., Strøm Johnsen, J., Vellan, E. E. & Gjessing, L. R.: Dietary treatment of cystinosis. *Acta Pediat Scand 57*: 409, 1968.

17 Wimmer, A.: Studies of the activation *in vitro* of glucuronyltransferase. *Biochim Biophys Acta 191*: 297, 1969.

18 Wolf, H. P.: 1-Phosphofructoaldolase. In H. U. Bergmeyer (ed.): *Methods of enzymatic analysis*. Academic Press, New York 1965, p. 732.

19 Wong, P. W. K., O'Flynn, M. O. & Inouye, T.: Micromethods for measuring phenylalanine and tyrosine in serum. *Clin Chem 10*: 1098, 1964.

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BILIRUBIN EXCRETION IN NEWBORN HUMAN INFANTS

I Unconjugated Bilirubin as a Possible Trigger for Bilirubin Conjugation

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Jaundice is observed in 30–80 per cent of all newborn infants (6, 12, 21) and the underlying hyperbilirubinemia reaches a maximum from the third to the sixth day in normal full term infants (2, 22)

No difference can be observed between the mature and premature infants in the amount of bilirubin present at birth (2) but prematures reach a much higher level of bilirubin than mature infants (4 13)

Bilirubin is unconjugated (UB) in cord blood both in normal fullterm and premature infants (5) because the enzyme bilirubin UDP glucuronyl transferase (BGT) (EC 2 4 1 17) is inactive at birth (17)

The first appearance of conjugated bilirubin (CB) in serum and thereby also of an active BGT has so far not been studied in detail The hypothesis that the hyperbilirubinemia of premature infants should be due to a later than normal activation of BGT (16) has neither been based on studies of the conjugating enzyme nor on the appearance of CB in the blood We therefore decided to study more closely the activation of bilirubin excretion in the newborn

This investigation also showed that the first appearance of CB in all types of hyperbilirubinemia so far studied is preceded by an accumulation of UB

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MATERIALS AND METHODS

Fullterm and premature infants

Ten fullterm and twentyfive premature infants were studied All infants received 1 mg vitamin K (menadion) intramuscularly immediately after birth

The fullterm infants were all born after normal pregnancies and had birthweights varying from 2680 to 4210 g Blood samples were taken from the heel every day for the first eight days of life

The premature infants had birthweights varying from 1140 to 2500 g none of them being small for date Two of these infants (birthweights 1140 and 1190 g respectively) died during the first week of life

Infants with Rh or ABO incompatibility were excluded from this material

Infants with erythroblastosis foetalis

This group consists of patients with erythroblastosis foetalis due to Rh immunization The anti D titers were determined in the mothers during pregnancy by the indirect Coombs tecniche (The Norwegian National Institute of Public Health)

(*a*) *Conjugated* and unconjugated bilirubin were measured in heel blood samples from 25 patients which according to the maximum anti D titer found in their mothers during pregnancy were divided into the following three groups

Group I	Max titer	0 – 128	7 cases
Group II	Max titer	256 – 512	8 cases
Group III	Max titer	1024 – 2048	10 cases

(*b*) Total bilirubin (TB) only was measured in 95 infants shortly after birth and they were divided according to the maximum anti D titer in their mothers during pregnancy into the following six groups

Group 1	Max titer	0 – 128	21 cases
Group 2	Max titer	128	8 cases
Group 3	Max titer	256	16 cases
Group 4	Max titer	512	16 cases
Group 5	Max titer	1024	22 cases
Group 6	Max titer	2048	20 cases

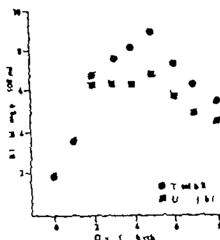


Fig 1 Total bilirubin and unconjugated bilirubin in serum from full term infants in the first week of life Each value is the mean of 10 sera

Three of the twenty infants in group 6 died from hydrops foetalis shortly after birth

Newborn infants delivered from jaundiced mothers

Five mothers all of them jaundiced during the last week of pregnancy were examined together with their newborn infants Three of the mothers (A B E R P A K W) had acute hepatitis one (A U) suffered from an inherited type of icterus (25) and one mother (M A) suffered from icterus of unknown cause Samples of blood were taken from the mothers by vein puncture and from the babies from the heel shortly after birth

Exchange transfusion

Exchange transfusion were performed according to the rule described by Aagenaes (2 24)

Bilirubin determinations

(*a*) Total bilirubin was determined according to Jendrassik & Gróf (15) modified by Fog (8)

(*b*) Unconjugated and conjugated bilirubin were determined according to the direct spectrophotometric method described by Fog & Bakken (10)

RESULTS

The amounts of total and unconjugated bilirubin found in normal and premature infants are shown in Fig 1 The difference between the two curves gives the amount of CB In the fullterm infants at birth TB varied from 1 1 to 2 2 mg with a mean of 1 7 mg per 100 ml The maximum TB concentration was reached on the fourth to sixth day with a mean of 8 6 mg per 100 ml on the fifth day

CB was not demonstrable at birth or on the first day of life but it appeared about 48 hours after birth then varying from 0 0 to 0 6 mg per 100 ml The maximum value of CB was reached on the third to sixth day after birth with 2 0 mg per 100 ml

In premature infants (Fig 2) the mean value of TB at birth was 2 0 mg per 100 ml The maximum was reached five to six days after birth with values from 11 to 19 mg per 100 ml CB usually appeared on the second to third day while the appearance of CB in two cases was delayed until the fourth day after birth CB increased to a maximum of 2.5 to 4 0 mg per 100 ml the third to sixth day

The mean values of CB in the different groups suffering from erythroblastosis foetalis are shown in Fig 3 In the heaviest immunized group all the infants had CB in their sera at birth In the moderately immunized group all had CB in their sera on the second day while four had CB even on the first day after birth None of the least immunized infants were able to conjugated bilirubin on the first day of life Four of them showed this ability after two days and all of them three days after birth Maximum concentrations of CB were reached in these groups on the third fourth and seventh day respectively The time of exchange trans-

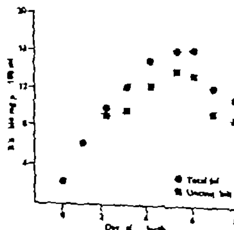


Fig 2 Total bilirubin and unconjugated bilirubin in serum from premature infants in the first week of life Each value is the mean of 23 sera

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BILIRUBIN EXCRETION IN NEWBORN HUMAN INFANTS

I. *Unconjugated Bilirubin as a Possible Trigger for Bilirubin Conjugation*

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Jaundice is observed in 30–80 per cent of all newborn infants (6, 12, 21) and the underlying hyperbilirubinemia reaches a maximum from the third to the sixth day in normal fullterm infants (2, 22).

No difference can be observed between the mature and premature infants in the amount of bilirubin present at birth (2), but prematures reach a much higher level of bilirubin than mature infants (4, 13).

Bilirubin is unconjugated (UB) in cord blood both in normal fullterm and premature infants (5) because the enzyme bilirubin UDP glucuronyl transferase (BGT) (EC 2.4.1.17) is inactive at birth (17).

The first appearance of conjugated bilirubin (CB) in serum and thereby also of an active BGT has so far not been studied in detail. The hypothesis that the hyperbilirubinemia of premature infants should be due to a later than normal activation of BGT (16) has neither been based on studies of the conjugating enzyme nor on the appearance of CB in the blood. We therefore decided to study more closely the activation of bilirubin excretion in the newborn.

This investigation also showed that the first appearance of CB in all types of hyperbilirubinemia so far studied, is preceded by an accumulation of UB.

This work was supported by grant no. E 303-8 from the Norwegian Research Council for Science and the Humanities.

MATERIALS AND METHODS

Fullterm and premature infants

Ten fullterm and twentyfive premature infants were studied. All infants received 1 mg vitamin K (menadion) intramuscularly immediately after birth.

The fullterm infants were all born after normal pregnancies and had birthweights varying from 2680 to 4210 g. Blood samples were taken from the heel every day for the first eight days of life.

The premature infants had birthweights varying from 1140 to 2500 g, none of them being small for date. Two of these infants (birthweights 1140 and 1190 g respectively) died during the first week of life.

Infants with Rh or ABO incompatibility were excluded from this material.

Infants with erythroblastosis foetalis

This group consists of patients with erythroblastosis foetalis due to Rh immunization. The anti D titers were determined in the mothers during pregnancy by the indirect Coombs tecnicbe (The Norwegian National Institute of Public Health).

(*a*) Conjugated and unconjugated bilirubin were measured in heel blood samples from 25 patients which according to the maximum anti D titer found in their mothers during pregnancy were divided into the following three groups:

Group I	Max titer 0 – 128	7 cases
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(*b*) Total bilirubin (TB) only was measured in 95 infants shortly after birth and they were divided according to the maximum anti D titer in their mothers during pregnancy into the following six groups:

Group 1	Max titer 0 – 128	21 cases
Group 2	Max titer 128	8 cases
Group 3	Max titer 256	16 cases
Group 4	Max titer 512	16 cases
Group 5	Max titer 1024	22 cases
Group 6	Max titer 2048	20 cases

(7 18). Hyperbilirubinemia due to UB might therefore be a reasonable trigger for the conjugating process. CB is found approximately 48 hours after birth in normal and premature infants. It is found even during foetal life and at birth in cases of erythroblastosis foetalis from heavily immunized mothers (Fig. 3) (1 8 9 11). It has never been shown that CB has appeared for the first time *in vivo* without a preceding hyperbilirubinemia due to UB. As shown for the erythroblastotic infants (Figs. 3 and 4) and infants born of icteric mothers (Table 1) a prenatal hyperbilirubinemia might be as useful a trigger for BGT as a postnatal hyperbilirubinemia for fullterm and premature infants.

The prenatal activation of BGT in the heaviest immunized infant may be explained by a trigger effect upon BGT from the high level of UB in these foetuses. The moderately immunized group did not have CB at birth, but these infants will have their bilirubin conjugating capacity earlier after birth than the least immunized group.

The findings in the moderately immunized group indicate that the concentration of bilirubin necessary to trigger BGT has been present prenatally for a too short period of time to finish the activation process. It seems therefore as if activation of BGT depends on the presence of a certain quantity of UB for a certain period of time.

The hyperbilirubinemia in premature infants must be due to other factors than later than normal activation of BGT (Figs. 1 and 2). In erythroblastotic infants, the extreme hyperbilirubinemia is partly due to a degradation of a greater than normal quantity of hemoglobin and partly due to the fact that CB cannot pass the placenta (19 20).

The theory of UB being the trigger for BGT is further supported by the finding of CB in the infants whose mothers were jaundiced during the last week(s) of pregnancy (Table 1). All the five infants had CB in their sera at birth and all the mothers had UB in their sera at a level above 1.8 mg per 100 ml. According to what has been found in monkeys (19) 1.5 mg UB per 100 ml gives a flux of bilirubin from the mother to the foetus. The foetuses from these icteric pregnant women have therefore been loaded with bilirubin before delivery.

All the infants described above got their first active BGT after a period of hyperbilirubinemia in utero or after birth. UB may then be the trigger substance for BGT even if the activating process is influenced by other substrates or hormones as phenobarbital (23) or oestrogens (14).

These results obtained by clinical studies and the hypothetical conclusions drawn are supported by earlier studies by rats where UB has been found to be a trigger substance for bilirubin conjugation (3).

SUMMARY

Studies of mature and premature infants, infants suffering from erythroblastosis foetalis and infants whose mothers were jaundiced during the last week(s) of pregnancy show that the time of activation of bilirubin UDP glucuronyl transferase may be related to an increase of unconjugated bilirubin in the foetus or in the newborn baby. The enzyme may be activated even before birth if the load of unconjugated bilirubin is sufficiently great.

REFERENCES

1. Bakken A F & Fog J. Bilirubin conjugation in the newborn. *Lancet I* 1280 1967
2. Bakken A F. *Bilirubinutskillelsens aktivering hos nyfødte.* Thesis Oslo 1969
3. — Effects of unconjugated bilirubin on bilirubin UDP-glucuronyl transferase activity in the liver of newborn rats. *Pediat Res 3* 205 1969
4. Billing B H, Cole P G & Lathe G H. Increased plasma bilirubin in newborn infants in relation to birthweight. *Brit Med J II* 1263 1954
5. Brown A K & Zuelzer W W. Studies on hyperbilirubinemia. I. Hyperbilirubinemia in the newborn unrelated to isoimmunization. *Amer J Dis Child 93* 263 1957
6. Davidson L T, Merritt K K & Weech A A. Hyperbilirubinemia of the newborn. *Amer J Dis Child 61* 958 1941
7. Dawkins M J R. Changes in glucose-6-phos

fusione were of no particular importance in determining the maxima (2)

The relationship between TB in the new born shortly after birth and the maximum anti D titer in the mother during pregnancy is shown in Fig 4 The least immunized infants had a mean concentration of TB of 3 mg per 100 ml at birth This level increases with increasing titer to about 6 5 mg per 100 ml in the heaviest immunized infants The mean serumbilirubin in the heaviset immunized group was found to be about 8 mg per 100 ml if the infants which died from hydrops foetalis were excluded

The levels of bilirubin in the jaundiced mothers and their babies are shown in Table 1 All five infants had CB varying from 0 2 to 3 5 mg per 100 ml in their sera at birth

DISCUSSION

The observations done in this work are based on the measurement of UB and CB in serum The method used (10) determines the UB by direct spectrophotometry The difference between the total bilirubin and UB is CB This pool of CB consists largely of bilirubinglucuronide, however other bilirubin conjugates may also be present The method has a methodological error less than 3% (2)

The appearance of CB at the same time after birth in mature and premature infants (Figs 1 and 2) seems to indicate that bilirubin conjugation has been started by something connected with delivery

Many enzymes which are inactive during foetal life are activated shortly after birth to take care of intermediate metabolites which during pregnancy have passed the placenta to be handled by the mother These metabolites including bilirubin accumulate when the umbilical cord is clamped It is generally accepted that an enzyme may be activated by an increase in its own substrate This has been shown to be valid in the newborn period too

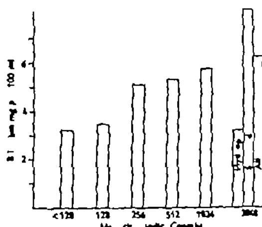


Fig 4 Total bilirubin at birth in different groups of erythroblastosis foetalis The infants are grouped according to the max anti D titer found in their mothers during pregnancy

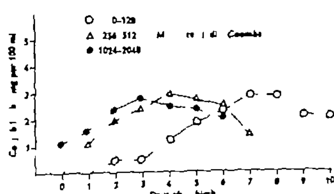


Fig 3 Conjugated bilirubin in sera from different groups of erythroblastosis foetalis during the first days of life The infants are grouped according to the max anti D titer found in their mothers during pregnancy

Table 1 *Total bilirubin and conjugated bilirubin in five mothers and their newborn babies at delivery*

All the mothers were jaundiced during the last week(s) of pregnancy

Patient		Total bilirubin (mg/100 ml)	Conjugated bilirubin (mg/100 ml)
A E	mother	5 8	1 3
	child	5 3	2 6
E R P	mother	9 1	5 4
	child	10 0	2 7
A K W	mother	7 5	3 7
	child	2 8	0 2
M A	mother	2 1	0 2
	child	3 4	0 9
A U	mother	6 5	2 3
	child	6 8	3 5

BILIRUBIN EXCRETION IN NEWBORN INFANTS

II Conjugated Bilirubin as a possible Trigger for Bilirubin Excretion

ARNE F BAKKEN

From the Paediatric Research Institute and Department of Paediatrics Rikshospitalet University of Oslo Oslo Norway

To be excretable unconjugated bilirubin (UB) has to be coupled to glucuronic acid by the enzyme bilirubin UDP glucuronyl transferase (BGT) (EC 2 4 1 17) This conjugated bilirubin (CB) is excreted from the liver cell into the bile by an active process (9 12) Such an active process is probably also present in the kidneys

The activation time of BF is an important factor in the hyperbilirubinaemia found in normal newborn babies (13) The liver bilirubin excretion mechanism has been found partly to depend on hormones (6)

The amount of CB within the liver cell may decrease not only because CB is excreted into the bile but also if CB is deconjugated by the enzyme β glucuronidase (EC 3 2 1 31)

It has been found that UB functions as a trigger for BGT in the newborn (1 2) In this paper it is shown how CB may trigger its own excretion in normal fullterm infants in premature infants and in infants suffering from erythroblastosis foetalis

Increasing amounts of CB are supposed to activate its own excretion in a similar way as UB activates BGT Both these functions have to be activated before the bilirubin is able to escape from the body through the bile or urine

This work was supported by grant No E 303-8 from the Norwegian Research Council for Science and the Humanities.

METHODS AND MATERIALS

Total bilirubin was determined according to Jendrassik & Gróf (8) modified by Fog (4) UB and CB were determined by direct spectrophotometry of diluted sera (5) All the blood samples were taken from the heel

Bilirubin in the urine was determined by a qualitative diazocolour reaction (Ictotest Ames Co) which is positive when bilirubin in the urine exceeds 0 05 mg per 100 ml

Full term infants

Ten normal full term infants from normal pregnancies and deliveries were studied during eight days after birth. Serum CB was determined if possible each day and samples of urine were collected and analyzed simultaneously with the blood

Premature infants

Twentyfive premature infants were studied (birth weights 1140-2900 g) Infants with Rh or ABO incompatibilities were excluded Twentythree infants survived the first week of life Samples of blood and urine were collected simultaneously if possible

Cases of erythroblastosis foetalis

The twentyfive infants suffering from erythroblastosis foetalis were grouped according to the maximal anti D titers found in their mothers during pregnancy (The titers determined by the indirect Coombs test by the Norwegian National Institute of Public Health.)

Group I	Max anti D-titer	0– 128	7 cases
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RESULTS

Fullterm infants

In normal fullterm infants CB first appeared in serum at 48 hours after birth at which time

phatase activity in liver and kidney at birth *Nature 191* 72 1961
8 Fog J Determination of bilirubin in serum as alkaline azobilirubin *Scand J Lab Clin Invest 10* 241 1958
9 — *Serumfarge og bilirubin i ikteriske sera* Thesis Oslo 1960
10 Fog J & Bakken A F Conjugated and unconjugated bilirubin determined in icteric sera by direct spectrophotometry *Scand J Lab Clin Invest 20* 88 1967
11 Harris L E Farrel F J Shorter R G Banner E A & Mathieson D R Conjugated serumbilirubin in erythroblastosis foetalis An analysis of 38 cases *Proc Staff Meet Mayo Clin 37* 574 1962
12 Hirsch A Die physiologische Ikterus bereitschaft des Neugeborenen *Z Kinderheilk 9* 196 1913
13 Hsia D Y Y Allen F H Diamond A K & Gellis S S Serumbilirubinlevels in the newborn infants *J Pediat 42 277 1953*
14 Hsia D Y Y Riabow S & Dowben R M Inhibition of gluguronyl transferase by steroid hormones *Arch Biochem Biophys 103* 181 1963
15 Jendrassik L & Gróf P Vereinfachte photometrische Metoden zur Bestimmung des Serum bilirubins *Biochem Z 297* 81 1938
16 Jirsova J Jirsa M & Janovsky M Importance of the quantitative determination of direct and indirect bilirubin in hemolytic disease of the newborn *Acta Paediat Scand 47* 179 1958
17 Lathe G H & Walker M The synthesis of bilirubin glucuronide in animal and human liver *Biochem J 70* 705 1958
18 Nemeth A M Initiation of enzyme formation by birth *Ann N Y Acad Sc III* 199 1963
19 Schenker S Bashore R A & Smith F Bi rubin disposition in foetal monkeys *in* J A B Bouchner & B H Billing (eds) *Bilirubin metab lism* Blackwell, Oxford and Edinburgh 19 p 199
20 Schmid R Bukinham S Wendills G H Hammaker L Bilirubin metabolism in th fetus *Nature 183* 1823 1959
21 Waugh T R Mercant hF T G & Maugha G B Blood studies on newborn direct and tot bilirubin Determination over 9-day period wi special reference to icterus neonatorum *Amer Med Sci 199* 9 1940
22 With T K *Biology of bile pigments* A Fra Hansen Copenhagen 1954 p 523
23 Yaffe S J Matsugawa T & Babah T E hancement of clucuronide conjugating capacity a hyperbilirubinemic infants due to apparent e zymeinduction by phenobarbital *New Eng Med 275* 1461 1966
24 Aagenæs Ø Behandling av erythroblasto foetalis *T Norsk Lægeforen 87* 1581 1967
25 Aagenæs Ø Van der Hagen C B & Refsu R Hereditary recurrent intrahepatic cholesta from birth *Arch Dis Child 43* 646 1968

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BILIRUBIN EXCRETION IN NEWBORN INFANTS

II Conjugated Bilirubin as a possible Trigger for Bilirubin Excretion

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The activation time of BE is an important factor in the hyperbilirubinemia found in normal newborn babies (13) The liver bilirubin excretion mechanism has been found partly to depend on hormones (6)

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It has been found that UB functions as a trigger for BGT in the newborn (1 2) In this paper it is shown how CB may trigger its own excretion in normal fullterm infants in premature infants and in infants suffering from erythroblastosis foetalis

Increasing amounts of CB are supposed to activate its own excretion in a similar way as UB activates BGT Both these functions have to be activated before the bilirubin is able to escape from the body through the bile or urine

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RESULTS

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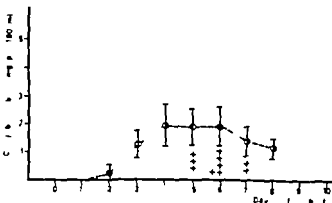


Fig 1 Conjugated bilirubin in the serum of ten normal fullterm infants (Means ± S D) The appearance of bilirubin in the urine in the single case is indicated by +

the eight infants had from 0 1 to 0 5 mg of CB per 100 ml (Fig 1) All the infants had CB in their sera 72 hours after birth and it increased to a maximum varying from 1 8 to 3 0 mg per 100 ml The amount then decreased to 0 1 to 1 8 mg per 100 ml eight days after birth The highest mean values 1 9 mg per 100 ml remained unchanged from the fourth to the sixth day

Bilirubin was detected in the urine for the first time from the fifth to the seventh day of life Early appearance of bilirubin in the urine corresponded to an early maximum of CB in the serum

Premature infants

None of the premature infants had CB in their sera until about 48 hours after birth (Fig 2)

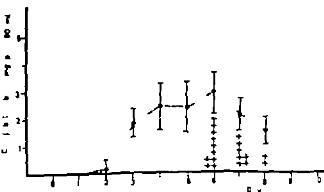


Fig 2 Conjugated bilirubin in the serum of twenty five premature infants (Means ± S D) The appearance of bilirubin in the urine in the single case is indicated by +

The mean values of CB in the 25 premature infants were 0 2 mg per 100 ml on the second to third day of life varying from 0 1 to 1 0 mg per 100 ml Maximum values of CB were reached on the third to seventh day of life with values rfom 2 5 to 6 0 mg per 100 ml The highest mean value of 3 1 mg per 100 ml was reached on the sixth day after birth The concentration of CB declined rapidly to about 1 5 mg per 100 ml on the eighth day of life

As for the fullterm infants bilirubin was detectable in the urine shortly after the maximum value of CB in the serum was reached Ten of the premature infants had bilirubinuria for the first time on the sixth day of life seven infants on the seventh day of life and two infants on the eighth day of life

Cases of erythroblastosis foetalis

The results in cases of erythroblastosis foetalis are presented in Table 1

The maximum amount of CB in the serum was reached on the fourth to seventh second to fifth and first to fourth day in the groups I II and III respectively The maximum values varied from 2 8 to 5 1 mg per 100 ml in group I 2 5 to 4 4 mg per 100 ml in group II and 2 5 to 4 5 mg per 100 ml in group III

While bilirubin was detectable in the urine for the first time on the sixth to ninth day in group I it was found on the third to sixth day in group II and on the second to fifth day in group III

DISCUSSION

The accumulation of CB shown in Figs 1 and 2 and in Table 1 indicates that the excretory function for this conjugate must be defective or immature at birth It therefore seems as if a factor different from BGT has to be activated before bilirubin can be eliminated from the body This factor becomes active at least from the fifth to the seventh day in mature and premature infants because the amount of CB in the blood from this time decreases and bilirubin from this time also can be detected in the

Table 1 *Mean values of conjugated bilirubin in different groups of erythroblastosis foetalis*
The first appearance of bilirubin in the urine in each case is indicated by +

Group	Max anti D titer in mother[a]	Days after birth										
		0	1	2	3	4	5	6	7	8	9	10
		(mg per 100 ml)										
I	0–128	0	0	0.4	0.8	1.1	1.8	2.2 +	2.8 + +	2.8	2.1 + + + +	2.0
II	256–512	0	1.0	1.8	2.3 + +	2.8 + +	2.6 + + +	2.4 +	1.4			
III	1024–2048	1.1	1.5	2.3 + + + +	2.7 + +	2.4 + + +	2.3 +	2.0				

[a] Anti D titer determined by the indirect Coombs test

urine It has been shown earlier that CB appears after birth as a possible response to an accumulation of UB and that hyperbilirubinemia for 24–28 hours was needed to activate BGT (2) It is reasonable to suppose then that accumulation of CB may act as a trigger for its own excretion in the same way as UB may trigger BGT

Table 1 shows that the appearance of bilirubin in the urine is related both to the amount of CB present in the blood and to the duration of the increase of CB Infants in group III for instance where the mothers had anti D titers exceeding 1024 must have had an increase of UB and even CB before birth Some of these infants started to excrete bilirubin as early as the second day after birth in contrast to the fifth day in normal mature infants

The excretion of CB seems to be an adaptive process in the same way as BGT is an adaptive enzyme From the figures and Table 1 it can be anticipated that a quantity of approximately 1 mg must be present in 100 ml serum for nearly three days before the excretion of CB is really effective

It is unknown if the liver starts to excrete CB at the same time as the kidneys However even if the liver should begin to excrete CB somewhat earlier then the kidneys the time difference can not be greater than a few hours since no decrease in serum CB is registered until bilirubin is found in the urine

The rate of disappearance of UB from the blood depends therefore not only on BGT as proposed by Matusada *et al* (10) but also on the excretion capacity for CB The importance of the enzyme β glucuronidase in decreasing the concentration of CB is not yet clear β glucuronidase is present in very small amounts at birth (7) and evidence is accumulating that the deconjugating capacity of this enzyme is adaptive and therefore may be triggered by an accumulation of CB (3 11)

SUMMARY

Studies of normal infants premature infants and infants suffering from erythroblastosis foetalis due to Rh immunization show that conjugated bilirubin (CB) may be able to trigger its own excretion mechanism This excretory function consists of two factors a liver cell excretion factor and a kidney excretion factor The role of the deconjugating enzyme β glucuronidase is still not clear as far as the bilirubin metabolism in the newborn is concerned

REFERENCES

1 Bakken A F Effects of unconjugated bilirubin on bilirubin UDP-glucuronyl transferase activity in the liver of newborn rats *Pediat Res* 3 205 1969

— Bilirubin excretion in newborn infants I Unconjugated bilirubin as a possible trigger for bilirubin conjugation *Acta Paediat Scand* 59 148 1970

3 Desmet J Bullens A M & Heirwegh K P M Histochemistry of bilirubin and conjugates *In* J A M Bouchier & B H Billing (eds) *Bilirubin metabolism* Blackwell Sc Publ Oxford & Edinburgh 1967
4 Fog J Determination of bilirubin as alkaline azobilirubin *Scand J Lab Clin Invest 10* 241 1958
5 Fog J & Bakken A F Conjugated and unconjugated bilirubin determined in icteric sera by direct spectrophotometry *Scand J Lab Clin Invest 20* 88 1967
6 Gartner L M The neonatal regulation of hepatic bilirubin excretion *in* J A M Bouchier & B H Billing (eds) *Bilirubin metabolism* Blackwell Sc Publ Oxford & Edinburgh 1967
7 Heringova A Koldovsky O & Jirsova J β glucuronidase activity in the serum of infants *Acta Pædiat Scand 55* 175 1966
8 Jendrassik L & Gróf P Vereinfachte Photometrische Metoden zur Bestimmung des Bilirubins *Biochem Z 297* 81 1938
9 Jirsova J Jirsa M & Janovsky M Importance of the quantitative determination of direct and indirect bilirubin in hemolytic disease of the newborn *Acta Pædiat Scand 47* 179 1958
10 Matsuda J & Shirahata T Bilirubin metabolism in the newborn *Lancet I* 938 1967
11 Raia S Histochemical determination of conjugated and unconjugated bilirubin in cholestatic liver tissue *in* J A M Bouchier & B H Billing (eds) *Bilirubin metabolism* Blackwell Sc Publ Oxford & Edinburgh 1967
12 Schenker S Disposition of bilirubin in the foetus and the newborn *Ann N Y Acad Sci 111* 302 1963
13 Schenker S Dawber N H & Schmid R Bilirubin metabolism in the foetus *J Clin Invest 43* 32 1964

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ANTHROPOMETRY IN PRIVILEGED ETHIOPIAN PRESCHOOL CHILDREN

CNU Report No 33

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In Ethiopia the physical growth of children in both rural and urban settlements has been studied (13 15 18 21 27) The findings of these reports give an almost unanimous picture the weight and height curves of infants very closely correspond to western standards during the first 3–5 months of life i e during the period of usually sufficient breast feeding after which the growth is retarded At the end of the infant year and during the following preschool period the children are 20–30% underweight and almost 10 shorter than their counterparts in Europe and America

The purpose of the present investigation was to study anthropometric data of a group of preschool purely Ethiopian children living under hygienic and socio-economic conditions approximately similar to those of average children in Europe and North America

MATERIAL

The children were recruited in two ways the mothers of many of them were members of an association of educated women in Addis Abeba but most of the children were recruited from kindergartens which charged expensive fees The basic selection principle was that both parents should have attended but not necessarily completed secondary school at least A total of 100 children were selected by this method and investigated

Most of the fathers and one third of the mothers had University or College degrees The fathers were employed as engineers, managers teachers army officers etc and most of the mothers were working full time usually as secretaries nurses social workers or teachers Their combined income could without doubt be considered outstandingly high by Ethiopian standards To judge by the appearance of those of their homes which were visited by the author by the availability of bathrooms private telephones refrigerators cars etc the living standard could be classed as the same or almost as high as that of many of the more privileged foreigners in Addis Abeba.

From an ethnic and religious point of view the material was heterogeneous all the main Ethiopian ethnic groups (Amhara, Tigre Galla Gourage) and religions (Orthodox Christians Moslems Catholics and Evangelical Christians) being represented

An exact birthdate was obtained for all children Thirty-five were 36 months of age or less the youngest being 4 months and 65 were from 37 to 60 months inclusive Fifty-one of the 100 children were boys

Information on birth weights was obtained for 47 children 44 of whom had been born in hospital Thirty of them had birth weights between 3 and 4 kg 10 below 3 kg and 7 over 4 kg Of those whose birth weights were under 3 kg four were prematurely born (weighing at birth 1 5 1 5 1 65 and 2 35 kg)

About two thirds of the mothers had started to give supplementary food when the child was 2 months old or younger Very few continued breast feeding for more than 4 months The supplementary food first given was always a high quality formula made in America or Europe The diet of the older children was according to information from the parents well balanced including eggs meat, vegetables and fruits Many parents gave vitamin pills regularly

Practically all children were vaccinated the most common vaccinations being BCG triple vaccine smallpox and poliomyelitis, but some were also vaccinated against measles and yellow fever

In the following the children of one of the above

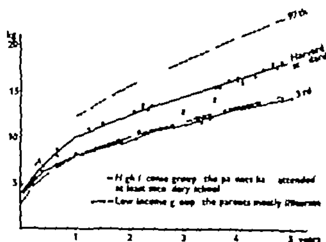


Fig 1 Body weight of privileged Ethiopian pre school children (*n* = 100) For comparison the mean weight curve for low income children (*n* = 432) in a typical Ethiopian region (Ijaji) (13) is given as well as the mean and the 3rd and 97th percentiles of the Harvard standard (22)

mentioned reports (13) will be referred to as "under privileged or low income children The study alluded to was carried out in a village Ijaji situated in a typical agricultural highland area 215 km west of Addis Ababa The subjects comprised 432 children of ages 0–5 years The methods used were the same as in the present study

METHODS

The nude weight of the children was recorded to the nearest 0 1 kg For children of about and under one year of age an infant scale accurate to 0 01 kg was used and for older children a beam scale accurate to 0 1 kg Both balances were repeatedly checked against known weights For children shorter than about 80–90 cm an infant length board was used For the rest of the children the standing height was measured (with steel tape attached to a wall) The length or height was recorded to the nearest full cm For the upper arm circumference the middle of the upper left arm was measured to the nearest 0 5 cm The triceps skinfold thickness was measured with a Harpenden caliper over the triceps muscle in the middle of the upper left arm and recorded to the nearest 0 2 cm The chest circumference was obtained as the circumference at the nipple line on mid inspiration and the head circumference as the greatest fronto-occipital circumference both being recorded to the nearest 0 5 cm The upper arm muscle circumference was calculated according to the formula recommended by Jelliffe (16) viz Upper Arm Muscle Circ = Upper Arm Circ — π Triceps Skinfold

The children were also examined clinically and when possible blood specimens were taken for hematological examination and amino acid screening (for methods see refs 5 12 and 13)

RESULTS

A General health condition

Generally the children under study appeared clinically healthy and in as good a condition as children seen in a Well Baby Clinic in for example, Sweden They were strikingly superior to children attending ordinary MCH Clinics in Ethiopia Clinical signs of malnutrition, such as wasted muscles and dyspigmented hair were never seen Apart from one child with evidence of active rickets, no signs of Vitamin A C or D deficiency were observed The mean hemoglobin value for 80 children 1–5 years of age was 13 0 g/100 ml (S D = 1 19) According to the altitude adjusted WHO lower anemia limit (12) 6 children (7 %) could be classified as having anemia in 2 of them the Hb was under 10 0 g/100 ml (one 8 1 and one 9 3 g%) and in 4 there was possible iron deficiency (MCHC below 27 %) The microsedimentation rate was above 20 mm in about 25 % of the children examined, possibly indicating present subclinical or previous infection in the otherwise apparently healthy subjects Considering that the corresponding figure for Ijaji pre school children was 80 % (13) the situation of the children under study can be regarded as relatively favorable in this respect also The amino acid ratio was 1 95 S D = 0 52 (*n* = 46) supporting the parents information that the children were receiving a well balanced protein diet

B Anthropometric findings

The *body weight* of each child under study is indicated by a black square on the Harvard growth chart of Fig 1 It can be seen that most of the weights fell between the 3rd and 97th percentiles of the Harvard standard (22) and all except 6 were above the average weight curve of low income children in Ijaji The weight for age distribution arranged according to Jelliffe s suggestion (16) is shown in Table 1 All children except 10 had weights for age between 80 and 120 % of the standard By comparison about half of the low income

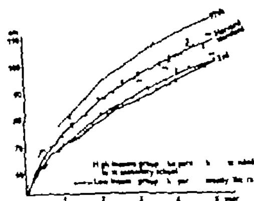


Fig 2 Heights (lengths) of privileged Ethiopian pre school children (n=100) For comparison the mean height curve for "low income" children (n=432) in a typical Ethiopian region (Ijaji) (13) as well as the mean and the 3rd and 97th percentiles of the Harvard standard (22) are also given

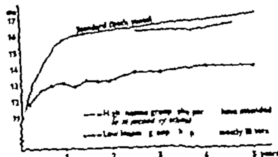


Fig 4 Mean upper arm circumference of privileged Ethiopian children 2–5 years old (n=83) For comparison the upper arm circumference curve for "low income" children (n=432) in a typical Ethiopian region (Ijaji) (13) as well as the mean of the standard of reference (Jelliffe adapted from Wolanski (16) are given

children 1–4 years of age in Ijaji had weights for age under 80% of the Harvard standard The average percentage of the standard of the privileged children below 3 years of age was 102 for children between 3 and 5 years 95% and for the whole group 97% (see Table 2)

The *height (length)* of each child under study is indicated by a black square in a similar way as for weight in Fig 2 Most of the children had a height (length) falling within the range of the 3rd and 97th percentiles of the Harvard standard (22) The height *for age* ratio lay between 91 and 110% of the standard for all except 5 of the children (Table 1) the average percentage of the Harvard standard for the whole group being 99% (Table 2)

The *weight for height* distribution is shown in Fig 3 and Table 1 the average percentage of the Harvard standard (16) being 99

The upper arm circumference for age was between 91 and 110% of the standard (Jelliffe adapted from Wolanski (16)) in 70 of *the 100 children (Table 1) the average per*centage of the standard being 98% (Table 2) Because the number of children under two years of age was relatively small only the three age groups 25–36 months 37–48 months and 49–60 months have been considered in Fig 4 (as well as in *Figs 5 and 6) It can be seen in*

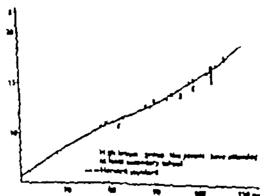


Fig 3 Weight for height of privileged Ethiopian pre school children (n 100) For comparison the mean of the Harvard standard (14) is also given

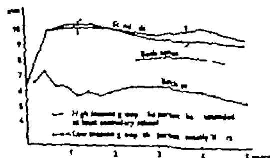


Fig 5 Mean triceps skinfold thickness of privileged Ethiopian children 2–5 years old (n=83) For comparison, the triceps skinfold curve for "low-income" children (n=432) in a typical Ethiopian region (Ijaji) (13), as well as the means of the standards of reference (Jelliffe adapted from Hammond Tanner & Whitehouse (16)) are also given

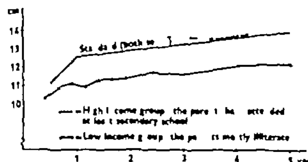


Fig 6 Mean upper arm muscle circumference of privileged Ethiopian children 2-5 years old ($n=83$) For comparison the upper arm muscle circumference curve for low income children ($n=432$) in a typical Ethiopian region (Ijaji) (13) as well as the mean of the standard of reference (Jelliffe calculated from the standard tables of upper arm circumference and triceps skinfold (16)) are also given

Fig 4 that the mean upper arm circumference of each of these three age groups of privileged children differs by only a few mm from the standard while the difference between the privileged and the underprivileged children (in Ijaji) is 5-8 times greater or about 2 5 cm

As seen in Fig 5 the *triceps skinfold thickness* of the three age groups of privileged children between 2 and 5 years was midway between that of the low income children in Ijaji and that of the standard (Jelliffe adapted from Hammond Tanner & Whitehouse (16)) The average percentage of the standard was 86% (Table 2) the distribution being wide and skewed

The *upper arm muscle circumference* for age was approximately normally distributed (Table 1) with an average of 100% of the standard (Jelliffe, calculated from the standard tables of upper arm circumference and triceps skinfold (16)) As seen in Fig 6 there was a marked difference between the privileged children and the children in the typical village of Ijaji

The *chest/head ratio* was initially not included in the examination form and therefore the head circumference was measured in only 78 and the chest circumference in only 46 of the 100 children The head circumference of privileged Ethiopian preschool children (Fig 7) seems to compare well with the standard of Watson & Lowrey (26) The chest/head ratio was less than one in 3 out of 15 children 1/-3 years of age and in 5 out of 31 children 3-5 years of age i e in 8 (17) of 46 children The average weight for age of these 8 children was 88% the average height for age 98% and the average upper arm muscle circumference 94 % of the standard

Teeth At the time of examination none of the 17 children of 2 years of age or less had all their deciduous teeth Four out of 9 children of ages 25–30 months and 8 out of 9 of ages 31–36 months had all their 20 milk teeth This was also true for all 65 children 37 months of age or older

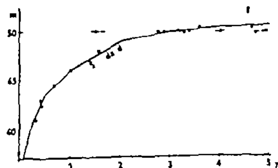


Fig 7 The head circumferences of privileged Ethiopian preschool children ($n=78$) For comparison the mean of the standard of reference (Watson & Lowrey (26)) is also given

DISCUSSION

To define *illness* we *must define health* It seems obvious that a knowledge of the normal should precede the knowledge of the abnormal (14) In probably very few aspects of child health and disease is the definition of normal more controversial than in the assessment of the nutritional status this in spite of the fact that *malnutrition in its different* forms and degrees is a leading pediatric problem in the developing areas of the world

One reason for the difficulties in classifying the severity of malnutrition is the lack of universally recognized anthropometric standards (4) This is essentially due to the fact that there is still no unanimous *agreement* on what is the

Table 1. *Some anthropometric parameters for 100 privileged preschool children in Ethiopia distributed to percentage of reference standard*

% of standard group	Wt/Age	Ht/Age[a]	Wt/Ht	Upper arm[b] circ./Age[b]	Triceps skin fold/Age[c]	Upper arm muscle[d] circ./Age
121	3	0	3	0	7	1
111–120	13	1	6	5	6	14
101–110	22	31	32	33	10	41
91–100	28	64	44	46	10	37
81–90	27	4	15	14	24	6
71–80	6	0	0	1	21	1
61–70	1	0	0	1	14	0
–60	0	0	0	0	8	0
	100	100	100	100	100	100

Standard of reference: Harvard (16, 22).
[a] Standard of reference: Jelliffe, adapted from Wolanski (16).
Standard of reference: Jelliffe, adapted from Hammond, Tanner & Whitehouse (16).
[d] Standard of reference: Jelliffe, calculated from the standard tables of upper arm circumference and triceps skinfold (16).

optimal development of the genetic potentiality or what is normal adaptation to environmental influences. To cite Garrow (9): Is the normal growth curve for a North American child necessarily optimum? Are children who are smaller than this necessarily malnourished?

The environmental influence on growth and physical development is well documented. That "privileged" "upper income" children are heavier and taller than underprivileged lowincome children of the same race has been shown in many parts of the world, e.g. India (25), West Africa (7), South Africa (17, 3), Jamaica (1) and North America (20). Also in his now classical study Greulich (10) proved that California Japanese children were taller and heavier than children in Japan. It is also common to relate the increase in mean weights and heights of children over the past decades and centuries (in the United States (11, 19), England (6), Jamaica (2), Japan (10) and other countries) to improved socio-economic conditions.

However, we must take into consideration possible genetic differences. The widely accepted belief that Mongolians are shorter by heredity than Caucasians is supported by the findings of Ashcroft *et al.* (3). They found that children of similar socio-economic status in Jamaica differed in weight and height apparently due to genetic factors. Chinese children were shorter and lighter than their European and African counterparts.

The findings of the present study support the above first cited reports that children from higher socio-economic classes have a physical growth superior to that of children of the same race living under poorer conditions. The children under study had approximately the same

Table 2. *Average percentage of reference standards of some anthropometric parameters for 100 privileged preschool children in Ethiopia distributed according to age groups*

Age (mo.)	No.	Wt/Age (%)	Ht/Age (%)	Wt/Ht (%)	Upper arm circ./Age (%)	Triceps skin fold/Age (%)	Upper arm muscle circ./Age (%)
4–36	35	102.1	100.3	101.4	100.0	86.3	104.6
37–60	65	94.6	97.8	97.9	97.2	85.9	100.0
4–60	100	97.2	98.7	99.[illegible]	98.2	86.1	101.6

For standards of reference see Table 1.

weight, height (length) and upper arm circumference as children in industrialized countries, and they were significantly superior to their socially underprivileged counterparts in towns and rural districts of Ethiopia

To shed further light on the possibility of a genetic factor, an attempt was made to relate *the heights (lengths) of the children to the* heights of their parents However very few parents were able or willing to cooperate The mean height of 14 fathers was 172 6 cm and that of 21 mothers 158 4 cm These values are above the mean heights of Ethiopian adults found by the ICNND team in 1958 (15) (166 5 cm and 154 5 cm respectively) but below Caucasian standards The number of parents, es*pecially of fathers is too small to allow any* decisive conclusions, but it would seem reasonable to interpret these findings as indicative of a secular trend towards increased growth in privileged families in Ethiopia

The youngest children under study were relatively generally slightly heavier taller etc than the older ones These differences do not seem to be of such an extent as to permit any detailed discussion but it may be relevant here to mention again that most of the mothers of the youngest children were members of the Association of Educated Women in Addis Ababa These women met weekly to listen to some lecturer or to discuss between themselves topics related to Baby Care Child Health Food Economy Family Planning and so forth and they were thus a progressive elite within the *total group of selected parents It should be* noted that almost all of these mothers were working full time, leaving their small children in the care of a nursemaid It is the author's opinion that among all the various environmental factors in early childhood influencing the physical development of the child the mother's educational level occupies a central position—regardless of whether she is a house *wife or professional* woman working full time

In one aspect, viz the triceps skinfold thickness the mean values of the children under study were well below the standard value for British children (adapted by Jelliffe (16)) A possible explanation for this "anthropometric discrepancy may be in accordance with a report from Tanzania (24) and recent findings in Ethiopia (8) that the body fat distribution of African children is different from that of Europeans

The *finding that 17% of the children* in whom the chest/head ratio was measured had ratios less than one, may indicate that this sign of malnutrition" should be used with some caution in nutrition surveys

SUMMARY

A cross sectional anthropometric survey of 100 Ethiopian children of ages 4–60 months was made Most of the children were recruited from kindergartens charging high fees, in Addis Ababa The basic selection principle was that *both parents should have attended at least secondary school* The living standard of the children studied could be classed as equal or almost equal to that of more privileged foreigners *living in Addis Ababa*

The body weight height (length) upper arm circumference triceps skinfold head circumference and chest circumference were measured In addition the weight for height and the upper arm muscle circumference were calculated

The results show that Ethiopian preschool children from higher socio economic classes *have a physical growth* superior to that of children belonging to the same ethnic groups but living under poorer conditions In fact, the children under study exhibited a rate of growth very close to the Western standard This might imply that the genetic potentiality for growth (at least during the first five years of life) of Ethiopian children corresponds well to that of Caucasian children

In one aspect viz the triceps skinfold thickness the mean values for the children under study were well below the standard of British children This anthropometric discrepancy

may possibly be due to a difference between the body fat distribution between Ethiopian and British children

REFERENCES

1 Ashcroft M T & Lovell H G Heights and weights of Jamaican children of various racial origins *Trop Geogr Med 4* 346 1964
— Changes in mean size of children in some Jamaican schools between 1951 and 1964 *W Ind an Med J 14* 48 1965
3 Ashcroft M T Heneage P & Lovell H G Heights and weights of Jamaican schoolchildren of various ethnic groups Amer J Phys Anthrop 24 35 1966
4 Bengoa J M Jelliffe D B & Perez C Some indicators for a broad assessment of the magnitude of protein-calorie malnutrition in young children in population groups *Amer J Clin Nutr 7* 714 1959
5 Bjornesjo K B Makonnen Belew & Zaar B Biochemical study of advanced protein malnutrition in Ethiopia *Scand J Clin Lab Invest 18* 591 1966
6 Clements E M B Changes in the mean stature and weight of British children over the past seventy years *Brit Med J II* 897 1953
7 Collis W R F Dema I & Lesi F E A Transverse survey of health and nutrition, Pankshin Division Northern Nigeria *W Afr Med J* 11 131 1962
8 Eksmyr R & Engstner G Skinfold thickness measurements in Ethiopian children To be published
9 Garrow J S Reply on letter by M Latham *Lancet I* 279 1967
10 Greulich W W A comparison of the physical *growth and development of American born and* native Japanese children *Amer J Phys Anthrop 15* 489 1957
11 Hathaway M L Heights and weights of children and youth in the United States. *Home Economic Research Report No 2 US Dept of Agriculture* Washington D C 1957
12 Hofvander Y Hematological investigations in Ethiopia *Acta Med Scand Suppl 494 1968*
13 Hofvander Y & Eksmyr R *An applied nutrition programme in an Ethiopian community* Part I (Baseline Data) Mimeographed paper Ethiopian Nutrition Institute Addis Ababa 1969
14 Illingworth R S *The normal child* J A Churchill Ltd London 1964 3rd edition p 7
15 *Interdepartmental* Committee on Nutrition for *National Defense (ICNND) Ethiopian Nutrition Survey* 1959
16 Jelliffe D B *The assessment of the nutritional status of the community* WHO Monograph Series No 53 1966
17 Kahn E & Freedman M L The physical development of a privileged group of African children *S Afr Med J 33* 934 1959
18 Mellbin T *et al* The nutritional status of children in five Ethiopian communities To be published
19 Meredith H V *Stature and weight of private* school children in two successive decades *Amer J Phys Anthrop 28* 2 1941
20 — Stature and weight of children of the United States *Amer J Dis Child 62* 909 1941
21 Nebiat Tefera Anthropometric studies in underprivileged Ethiopian preschool children To be published
22 Nelson W E *Textbook of Pediatrics* Saunders Philadelphia 1964 8th edition
23 Phillips H T *Some social and ethnic variations* in the physique of South African nursery school children *Arch Dis Child 28* 226 1953
24 Robson J R K Skinfold thickness in apparently normal African adolescents *J Trop Med Hyg 6* 209 1964
25 Udani P M Physical growth of children in different socio-economic groups in Bombay *Indian J Child Health 12* 593 *1963*
26 Watson E H & Lowrey G H 1962 quoted in D B Jelliffe *The assessment of the nutritional status of the community* WHO Monograph Series No 53 p 71 1966
27 Woodruff C W & Hoerman K Nutrition of infants and preschool children in Ethiopia. *Public Health Reports* Vol 75 No 8 724 1960

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Table 1 *Cases of central nervous leukemia treated intrathecally with cytosine arabinoside*

No	Initials	Age at time of manifestation of CNS leukemia (years)	Time from onset of disease (months)	Phase of the disease in which CNS leukemia developed	Type of CNS leukemia	Treatment with methotrexate before appearance of CNS leukemia: Total dose mg/kg body weight	Interval after last admin (days)
1	M S	13	4	II relapse	Meningeal	4 0	60 0
	M S	13 5	29	IV relapse	Cerebral	10 0	X
	M S	13 5	30	IV relapse	Cerebral	10 0	6 0
2	W J	6	11	I remission	Meningeal	14 48	16 0
3	T J	6	16	IV relapse	Meningeal	19 85	6 0
4	J B	11	4	I remission	Meningeal	7 87	X

X Administration being continued

tration of methotrexate The blastic phases of the disease were treated with prednisone in conjunction with 6 mercaptopurine or methotrexate and vincristine In the third bone marrow relapse routine tap of the cerebrospinal fluid revealed meningeal leukemia which at that time was not manifested by any neurologic symptoms Methotrexate was injected intrathecally (Table 1) After the cerebrospinal fluid returned to normal the drug was continued at longer time intervals (14 28 days) to prevent a relapse of the central nervous system

The patient was n xt hospitalized because of the presence of pleocytosis (56 paralymphoblasts per mm³) during control CSF examinations The general condition of the child was good and physical examination did not reveal any abnormalities Moderate paralymphoblastosis was found in the myelogram (22%) The bone marrow relapse was treated with dexamethasone Because of possible resistance to the drugs used until then treatment with cytosine arabinoside (cytarabine¹) was begun After three intravenous injections in doses of 2 mg/kg/72 hours remission in the bone marrow was obtained

In three consecutive examinations of the CSF pleocytosis remained on a steady level and gram negative bacilli belonging to the family Achromobacteriaceae were isolated Tetracycline was therefore given orally and methotrexate was injected intrathecally On this treatment the child's condition deteriorated headaches vomiting and meningeal signs appeared followed by encephalic symptoms which presented a threat to the patient's life Pleocytosis rose to 2666/mm³ (Table 2) In view of a resistance to methotrexate cytosine arabinoside was injected intrathecally in a dose of 20 mg Two days after the injection the boy's condition improved and after three intrathecal injections of the drug pleocytosis dropped to a normal value

During the marrow and cerebromeningeal remission speech disorders appeared suddenly with subsequent convulsions which were repeated three times This syndrome was connected with changes in excretion of the urinary ions and marked alteration of the EEG (Table 3 Figs 1 and 2) This indicated

¹ Supplied by Upjohn Co Kalamazoo U S A

Table 2 *Methotrexate and cytosine arabinoside administered intrathecally*

No	Initials	Methotrexate: No of injections	Methotrexate: Total dose in mg/kg body weight	Methotrexate: Pleocytosis after treatment	Cytosine arabinoside: No of injections	Cytosine arabinoside: Total dose in mg/kg body weight	Cytosine arabinoside: Pleocytosis after treatment
1	M S	3	0.5	10	—	—	—
	M S	3	0.3	2666	3	1.2	6
	M S	—	—	—	2	0.8	205[a]
2	W J	4	0.1	96	4	2.2	7
3	T J	3	0.3	305	4	3.8	34[b]
4	J B	9	1.6	131	2	0.93	8

[a] Described in the text
[b] After one injection of methotrexate pleocytosis returned to normal.

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CYTOSINE ARABINOSIDE ADMINISTERED INTRATHECALLY IN CEREBROMENINGEAL LEUKEMIA

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Involvement of the central nervous system occurs in acute lymphoblastic leukemia in about 1/3 of cases (3 10) The basic treatment of the disease in this localization consists in the intrathecal administration of methotrexate In addition in refractory cases of leukemia in the central nervous system X ray therapy is employed (2, 12) A number of communications have reported the use of cytosine arabinoside and l asparaginase in acute lymphoblastic leukemia localized in the CNS (1 9 12) These efforts to modify the treatment of meningeal leukemia indicate that not all cases respond favorably to treatment with methotrexate and that they differ in susceptibility to therapy and in the course of their development

Observations at our clinic incline us to believe that cases of leukemia of the central nervous system are not a homogeneous group On the basis of the clinical symptoms susceptibility to therapy course biochemical data and EEG findings we have distinguished two forms of this localization of leukemia 8)

The much more frequent meningeal form of leukemia of the central nervous system is characterized by a discrete mild course and by a lack of symptoms of pronounced disorder of *the functioning of the brain susceptibility to* intrathecal therapy and good immediate prognosis The increased pleocytosis with or without other marked signs of meningeal irritatio may represent the only symptoms of CNS i volvement

The second form is marked by cerebr symptoms, less pronounced pleocytosis fu minant course and poor immediate prognos despite regression of the changes in the cer brospinal fluid This form has been called cer bral leukemia The most striking difference be tween the meningeal and cerebral forms is th length of time of survival from diagnosis (meningeal leukemia to death In 24 cases (the meningeal form the mean survival time wa 12 5 months and in 3 cases of the cerebr form between several hours and 11 day Having distinguished two forms of leukemia (the central nervous system an attempt wa made to modify therapy in the cerebral form as described below

CASE REPORT

In an 11 year old boy M S acute lymphoblast leukemia was diagnosed on April 5 1965 After si weeks of treatment with prednisone 6 mercaptopurin and special diet (6) bone marrow remission was ob tained followed shortly by complete remission b June 8 1965 In the course of 30 months treatmen three relapses of the disease occurred The first re lapse was localized in the testes and was treated wi roentgen therapy and the next two relapses con cerned the bone marrow In the first two remission 6 mercaptopurine was administered and in the la

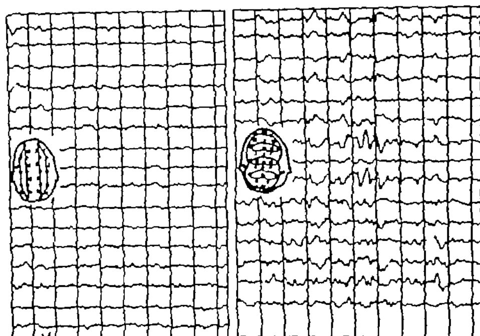


Fig 2 EEG in the child M S with the cerebral form of leukemia of the central nervous system Series of high voltage slow waves in the deep leads from the occipitoparietal region In the parasagittal leads the tracing is on the boundary of normal

bral form of leukemia were quickly fatal (8) In the patient reported in this paper administration of cytosine arabinoside combined later with X ray therapy produced a remission

Characteristic in this case was the simultaneous occurrence of clinical cerebral symptoms and a deep" EEG alteration connected with a disturbed ionic pattern of the urine

An analogy to the behaviour of the urine in TB encephalomeningitis described previously (4 7) pertaining to development of cerebral symptoms and occuring simultaneously with the regression of inflammatory signs in CSF should be mentioned

In the reported case of the CNS leukemia urinary ionic changes (Table 1) were accompanied by cerebral symptoms and an alteration in the deep EEG pattern (Fig. 2) *This might* indicate nonspecificity of the urinary changes in cerebral disorders

In three other cases of central nervous system leukemia refractory to treatment with methotrexate cytosine arabinoside proved effective (Tables 1 and 2) These cases were classified by us as the meningeal form of central nervous system leukemia Resistance to treatment in these cases manifested itself by a persistence of pleocytosis in the cerebrospinal fluid whereas in the case of cerebral leukemia described above (M S) lack of susceptibility to methotrexate was manifested by the appearance of central nervous system symptoms threatening the patient s life

Why the population of leukemic cells in the cerebrospinal fluid became insensitive to intrathecal methotrexate is not clear In all the reported cases the drug had been given before resistance to intrathecal methotrexate developed The total doses of methotrexate administered before the emergence of resistance to the drug are set out in Table 1 The drug was administered as a supportive measure during remissions of either bone marrow or meningeal leukemia, and the intervals in its administration were short Intrathecal administration of cytosine arabinoside was not attended by any

Table 3 *The excretion of urinary ions and the form of CNS leukemia in boy M S*

Form of CNS leukemia	Na mEq/min/1 73 m²	K	Cl	Na/K	Cl/Na
Meningeal	0 080	0 041	0 089	1 95	1 11
Cerebral	0 009	0 049	0 070	0 18	7 78
Cerebral	0 00045	0 0007	0 0005	0 64	1 11
Normal values (5)	0 165 ±0 058	0 037 ±0 017	0 188 ±0 064	5 00 ±2 04	1 26 ±0 16

that the cerebral form of CNS leukemia had developed In view of the suspected blastic proliferation in the cerebral tissues radiotherapy of the skull was performed resulting in a regression of all neurologic symptoms

During the third relapse of cerebromeningeal leukemia intrathecal injection of cytosine arabinoside proved ineffective and normalization of the regression of clinical symptoms was achieved by increasing the intrathecal dose of methotrexate to 0 2 mg/kg body weight The child had two further bone marrow relapses with an enlargement of the kidneys during the last one The boy died 35 months after the beginning of treatment

Autopsy revealed extensive leukemic infiltrations of the internal organs mainly existing in the kidneys generalized hemorrhagic diathesis cerebral oedema and internal hydrocephalus

DISCUSSION

In the reported case of acute lymphoblastic leukemia, there was a pronounced tendency to extramedullary relapses of the disease, manifested by involvement of the testes, by localization in the CNS on 3 occasions and renal tumors in the terminal stage of the disease Cerebro meningeal leukemia was detected twice during routine lumbar taps and clinical symptoms appeared later Asymptomatic course was observed in the early phase of the meningeal form at the beginning of relapse despite the existing cerebrospinal fluid changes On the basis of the clinical symptoms and the results of laboratory examinations the course of the CNS relapses was classified as the meningeal form Later cerebral changes developed with disturbances in the ions of the urinary excretion and an alteration of the EEG trace

Three previously observed cases of the cere

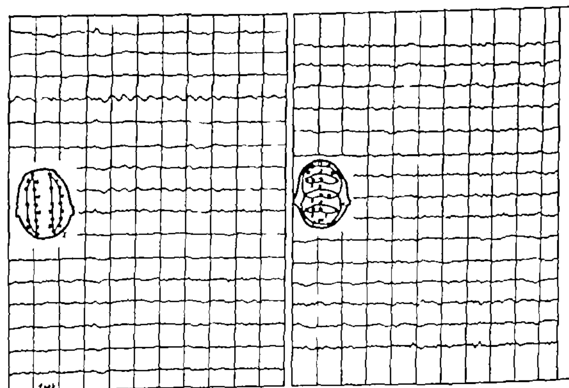


Fig 1 EEG in the child M S with the meningeal form of leukemia of the central nervous system Numerous delta waves in the parasagittal leads from the occipitoparietal region especially on the right side In the deep leads the tracing is on the boundary of normal

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LONGITUDINAL STUDIES OF INTELLECTUAL AND BEHAVIORAL DEVELOPMENT IN CHILDREN WITH CONGENITAL HEART DISEASE

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This is the final paper in a series (5 6 9) which reports the results of a five year developmental study comparing the intelligence and adjustment of children with cyanotic congenital heart disease those children with acyanotic cardiac disease and normal children Previous papers have not dealt with changes during the five year study period

Possible effects of intervening cardiac operation upon the development of children with congenital heart disease are of primary interest More than 40% of the children in the cardiac group underwent operation during the course of the investigation and so we were able to compare the development of operated and non operated children We studied changes in physical capacity intelligence and adjustment of the child and also changes in the mother's attitude toward her child the extent of her anxiety and her need to protect and pamper him

METHODS

Sample

A total of 119 children were studied Forty nine boys and 49 girls with congenital cardiac malformation marked cyanosis and physical handicap (Group 4) were compared with [illegible] boys and 48 girls with congenital cardiac disease handicap but without cyanosis (Group 3) Group 1 consisted of 31 normal siblings closest in age to the congenitally handicapped patients while Group 2 was composed of 40 normal children chosen at random from a well baby clinic Patients in whom the heart disease was one manifestation of a general syndrome which might have included mental retardation were excluded from the study

Assessment of intelligence and developmental status

Each subject was administered a developmental and/or intelligence test appropriate to his age Children under 2 ½ to 3 years received both the Cattell Infant Intelligence Scale and the Gesell Developmental Schedules From age 2 ½ to 3 the revised Stanford Binet Scale Forms L and M were used Forms were alternated in order to minimize practice effects Children were tested at 6 month intervals below the age of 3 and at yearly intervals above 3 years of age Ideally children first tested at age 6 months were re tested with the Gesell and Cattell at ages 1 1 ½ 2 and 2 ½ years At age 3 Stanford Binet Form L and at age 4 Stanford Binet Form M were administered Inasmuch as children with cardiac disease were added to this study in order of their first appearance at the hospital test sequence age sequence and number of tests vary among children When cardiac operation intervened psychological tests were performed preoperatively 3 to 6 months postoperatively and then at yearly intervals until stable results were obtained or until the project was completed

The child's behavior during the test was rated for attention willingness self-confidence social confidence and physical level of activity

Semi-structured interviews with the mother were conducted at the time of each test by the examining psychologist Seven point rating scales were devised for maternal attitude child adjustment and physical management variables which could be rated In view of the longitudinal nature of the study the psychologists usually were aware of the patient's course including the retesting and the personal interviews whether or not operation had been performed

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toxic symptoms It may be concluded that cytosine arabinoside administered intrathecally extends the range of drugs used in the treatment of leukemia of the central nervous system

SUMMARY

Two clinical forms of CNS leukemia in children may be distinguished the meningeal and the cerebral The following features constitute the differences between these two forms the clinical symptoms the number of cells in the CSF EEG trace, urinary findings and survival time The main difference, however is revealed in the response to routine therapy

Therefore some attempt has been made to vary the therapy Arabinoside of cytosine was also used intrathecally in the meningeal form of CNS leukemia in cases with a small degree of sensitivity to methotrexate therapy

REFERENCES

1 Boiron M Jacquillat Cl Weil M Tanzer J & Bernard J L asparaginase *Presse Med 76* 2099 1968
2 Evans A B D Angio G J & Mitus A Central nervous system complications of children with acute leukemia An evaluation of treatment methods *J Pediat 64* 94 1964
3 Haghbin M & Zuelzer W W A long term study of cerebrospinal leukemia *J Pediat 67* 23 1965
4 Halikowski B The pathogenesis and the course of tuberculous encephalo meningitis (In Polish) *Gruzlica 22* 461 1954
5 — Insuffisance du tubule renal au cours de l encephalo meningite tuberculeuse des enfants et son importance pour le pronostic *16 eme Congrès Ped Lang Franc* Paris 1957 p 29
6 Halikowski B Armata J & Garwicz, S Low protein purine free diet in treatment of acute leukemia in children preliminary communication *Brit Med J I* 519 1966
7 Halikowski B Klita S & Nawarecki B The correlation of the Na, K and Cl urine content ions pH volume and concentration ratio of the urine to the "cerebral" symptomatology in Tb *meningitis in children X Intern Congr Ped* Lisboa 1962 p 262
8 Halikowski B Wyszkowski J Armata J Garwicz, S Cyklis R & Brynak C The "meningeal and cerebral" forms of leukemia of the central nervous system in children (In Polish) *Pediat Pol 43* 145 1968
9 *Howard J P Albo V & Newton Jr W A* Cytosine arabinoside Results of a cooperative study in acute childhood leukemia *Cancer 21* 341 1968
10 *Hyman C B Bogle J M Brubaker* Ch A & Williams K Central nervous system involvement of leukemia in children I Relationship to systemic leukemia and description of clinical and laboratory manifestation *Blood 25* 1 1965
11 Kozlowska J & Rokicka Milewska R The infiltrations of the central nervous system in the course of acute leukemia in children (In Polish) *Pediat Pol 42* 919 1967
12 Najean Y Jacquillat Cl Weil M Tanzer J & Boiron M Orientation actuelles de la chimiothérapie des leucemies aigues *Vie Med 48* 949 1967

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Table 2. *IQ means for cardiac groups*
Unadjusted means for first and last tests and means at last test adjusted for six covariates

Group (1)	N (2)	First test: Mean (3)	First test: $s_{\bar{x}}$ (4)	Last test: Mean (5)	Last test: $s_{\bar{x}}$ (6)	Last test: Adjusted mean (7)	Last test: $s_{\bar{y}}$ (8)
Acyanotic							
Operated 35	31	102.5	2.5	103.4	2.0	99.8	2.2
Nonoperated 35	45	105.2	3.0	109.9	1.7	105.1	2.0
Cyanotic							
Operated 45	31	99.4	2.7	104.8	2.1	107.1	2.5
Nonoperated 45	43	94.6	3.0	95.1	1.7	101.1	2.1

the rankings of the IQ's we observed at the time of the last test cannot be explained by differences in age composition of the groups or by differences in economic level (as measured by the education of the father).

It was further found that the inclusion of incapacity as a covariate always resulted in changes in the rank orders of the adjusted means. Exclusion of incapacity always left the rankings the same as those of the unadjusted means (Table 2, Column 5). Whenever incapacity was included, the ranking of the means was as in Table 2, Column 7.

Covariance analyses in a survey are difficult to interpret. An explanation of the adjusted means in Table II might be as follows: looking separately at the acyanotic and the cyanotic groups. Differences in incapacity, age and initial IQ cannot explain more than part of the difference observed between final IQ for the 45 and 45 subgroups, so that we might possibly attribute the remaining difference (107.1 to 101.1) to the operation.

Similarly, in comparing the *two subgroups* of the acyanotic children, we see that the difference in final IQ (103.4 to 109.9) *cannot* be attributed to initial IQ or incapacity. The reason for this difference is not clear, but factors involved in selection for operation or effects of operation itself may be involved.

In comparing the cyanotic children with the acyanotic, one should be especially *cautious*. *We* see here that in the event that an operated cyanotic child has initial incapacity and initial IQ equal to an unoperated acyanotic, his IQ on the last test might be expected on the average to be higher than that of the acyanotic child. Since cyanotic children often have physical handicaps which give spuriously lower developmental quotient on tests *performed* at a lower age, statistical adjustment raises their final IQ. In other words, the cyanotic children did relatively well on final IQ considering their age, initial IQ and their incapacity.

Initial low IQ *in the* cyanotic children is *related*, at least in part, to their incapacity. *Therefore, the* increase in IQ *in this group* following successful *operation* with its attendant *clinical* improvement is readily predicted and explained. The lack of IQ *increase* in the unoperated cyanotic children is expected in a similar way. In the acyanotic children, initial handicap is less and IQ at first testing is *therefore* closer to the true potential of the individual.

The tendency *to increase in IQ* in the unoperated acyanotic children may be explained by *a combination of initial* condition and selection. This subgroup consists of children who entered the study with a mean incapacity slightly lower than that of the *other* acyanotic subgroup. Nothing untoward occurred during their period on the project to necessitate an *operation*; they did relatively well.

To study the changes in IQ for different diagnostic categories within the cyanotic

Table 1 *IQ means for first and last tests and mean changes for normal and cardiac groups*

Group (1)		N (2)	Mean at first test (3)	Mean at last test (4)	Difference (5)	SE of difference (6)	t (7)
Well baby	1	39	112 8	114 5	1 7	2 12	80
Sibling	2	68	110 3	113 9	3 6	1 30	2 77[a]
Acyanotic operated (S)	3S	35	102 3	103 3	9	1 66	54
Acyanotic non operated (S̄)	3S̄	45	105 2	109 9	4 7	2 40	1 96
Cyanotic operated	4S	33	99 9	105 2	5 3	1 89	2 79[a]
Cyanotic non operated	4S̄	45	94 6	95 1	45	1 71	26

Differences between mean IQ s first and last test are significant using a single test level of 0 05

A complete description of methods for assessing child adjustment and maternal attitude appears in Linde *et al* (5) For a more detailed account of sampling procedures and the assessment of physical status refer to Linde *et al* (7)

Methods of analysis

Preliminary analyses included all data collected during the course of the investigation Longitudinal developmental measures were graphed and studied for information from all tests Because of (1) variations in both the number of tests per child and the length of time each child was studied and (2) the need to establish a basis for a meaningful comparison between surgical and non surgical groups the first and last test measures were selected for final analysis The data were analyzed in several ways (1) Group means of variables measured at the first and last tests were compared within each group studied (cyanotic without surgery 4S̄ cyanotic with surgery 4S acyanotic without surgery 3S̄ acyanotic with surgery 3S siblings of cardiacs 2 well babies 1) (2) A comparison among all groups of changes in the means of attitudinal and intellectual variables between the first and last tests was made (3) Covariance analyses of IQ on the last test for surgical and non surgical cardiac groups were made on a selected set of covariates (incapacity at the first test incapacity at the last test the square root of age at the first test the square root of age at the last test education of the father at the first test and IQ measured on the first test) This analysis makes it possible to determine whether the differences observed among the groups in IQ measured on the last test might be explained by differences among any of the covariates

DISCUSSION

Intellectual development

A comparison of changes in IQ from the first to the last test within groups (Table 1) shows that all groups made some gains in mean IQ a finding which may be related to improvement with practice (1) The major gain was in the cyanotic group with intervening surgery the only statistically significant change among cardiac groups Normal siblings of children with cardiac disease also showed gains However when increases in IQ for operated groups were compared with increases in IQ for non operated groups none of the comparisons was statistically significant

The failure of the operated acyanotic children to improve appreciably and the almost significant gain in IQ for the acyanotic group without operation ($t = 1\ 95$) were unanticipated findings An effort was made to understand and interpret the entire pattern of changes among the cardiac groups For this purpose, a covariance analysis was performed Such an analysis can help to determine if the observed changes in IQ can be explained in terms of the initial composition of the groups or in terms of other variables measured at the time of the last test

A covariance analysis was performed with IQ on the last test as the dependent variable and with age education of the father IQ and incapacity at the time of the first test and age and incapacity at the time of the last test as covariates Various combinations of the six covariates were used in an attempt to understand the adjusted means of final IQ

It was seen first of all that the only covariates whose inclusion or exclusion made any difference in the analysis were IQ at the first test and incapacity either at the first or last test Age and education of the father made no difference This gives us some assurance that

Table 2 *IQ means for cardiac groups*

Unadjusted means for first and last tests and means at last test adjusted for six covariates

Group (1)	N (2)	First test Mean (3)	S E (4)	Last test Mean (5)	S E (6)	Adjusted mean (7)	S E (8)
Acyanotic							
Operated 3S	34	102.5	2.5	103 4	2 0	99 8	2 2
Nonoperated 3S	45	105 2	3 0	109 9	1 7	105 1	2 0
Cyanotic							
Operated 4S	31	99 4	2 7	104 8	2.1	107 1	2 5
Nonoperated 4S	45	94 6	3 0	95 1	1 7	101 1	2 1

the rankings of the IQ s we observed at the time of the last test cannot be explained by differences in age composition of the groups or by differences in economic level (as measured by the education of the father)

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Similarly in comparing the two subgroups of the acyanotic children we see that the difference in final IQ (103 4 to 109 9) cannot be attributed to initial IQ or *incapacity* The reason for this difference is not clear but factors involved in selection for operation or effects of operation itself may be *involved*

In comparing the cyanotic children with the acyanotic one should be especially *cautious* We see here that in the event that an operated cyanotic child has initial incapacity and initial IQ equal to an unoperated acyanotic his IQ on the last test might be expected on the average to be higher than that of the acyanotic child Since cyanotic children often have physical handicaps which give spuriously lower developmental quotient on tests *performed* at a lower age statistical adjustment raises their final IQ In other words the cyanotic children did *relatively well* on final IQ considering their age initial IQ and their incapacity

Initial low IQ in the cyanotic children is *related at least* in part to their incapacity Therefore the increase in IQ in this group following successful operation with its attendant clinical *improvement* is readily predicted and explained The lack of IQ increase in the unoperated cyanotic children is expected in a similar way In the acyanotic children *initial handicap* is less and IQ at first testing is therefore closer to the true potential of the individual

The tendency to increase in IQ in the unoperated acyanotic children may be explained by a combination of initial condition and selection This subgroup consists of children who entered the study with a mean incapacity slightly lower than that of the other acyanotic subgroup Nothing untoward occurred *during their period* on the project to necessitate an operation they did relatively well

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Acyanotic operated (S)	3S	35	102 3	103 3	9	1 66	56
Acyanotic non operated (*S*)	3*S*	45	105 2	109 9	4 7	2 40	1 95
Cyanotic operated	4S	33	99 9	105 2	5 3	1 89	2 8*
Cyanotic non operated	4*S*	45	94 6	95 1	45	1 71	26

* Differences between mean IQ s first and last test are significant using a single test level of 0 05

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DISCUSSION

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Table 4 *Psychological adjustment variables*
Means at first and least tests and mean changes[a]

Group (1)		N (2)	Mean at first test (3)	Mean at last test (4)	Difference (5)	s.e. of difference (6)	t (7)
(a) *General adjustment*							
Well baby	1	37	2 84	2 81	- 03	17	- 17
Sibling	2	61	2 88	3 13	25	16	1 43
Acyanotic operated	35	34	3 15	3 09	- 06	20	- 30
Acyanotic nonoperated	35	46	2 70	2 98	28	16	1 78
Cyanotic operated	45	34	3 62	2 79	- 83	23	- 3 62[b]
Cyanotic nonoperated	45	48	2 96	3 25	29	16	2 13[b]
(b) *Willingness*							
Well baby	1	37	2 35	11	24	15	- 1 63
Sibling	2	67	2 36	2 21	- 15	13	- 1 49
Acyanotic operated	35	38	2 34	2 53	19	14	1 35
Acyanotic nonoperated	35	51	2 37	2 41	04	13	30
Cyanotic operated	45	34	2 79	2 21	- 58	18	- 3 23[b]
Cyanotic nonoperated	45	51	2 35	2 43	12	17	- 72
(c) *Social confidence*							
Well baby	1	38	2 61	2 39	- 22	19	- 1 13
Sibling	2	68	2 28	2 41	13	12	1 10
Acyanotic operated	35	38	2 55	2 60	05	19	26
Acyanotic nonoperated	35	51	2 43	2 43	00	19	0
Cyanotic operated	45	34	3 03	2 59	- 44	20	- 2 21[b]
Cyanotic nonoperated	45	51	2 61	2 57	- 04	16	- 26
(d) *Attention*							
Well baby	1	38	2 66	2 47	- 19	20	- 93
Sibling	2	68	2 54	2 40	- 14	15	- 94
Acyanotic operated	35	38	2 79	2 61	- 18	20	- 91
Acyanotic nonoperated	35	51	2 55	2 53	- 02	17	- 11
Cyanotic operated	45	34	3 03	2 09	- 94	18	- 5 12[b]
Cyanotic nonoperated	45	51	3 06	2 71	- 35	18	- 1 91

[a] Negative change signifies improvement
[b] Mean changes from first to last test are significant using a single tail level of 0 05

changes in the combined non-operated groups (1 + 2 + 35 + 45) it is again clear that only the operated cyanotics make significant gains in general adjustment, attention and willingness

Although ratings of the child's anxiety were obtained from the parent interview many mothers were unable to determine if the child was anxious or not particularly in the younger children Consequently data do not permit any statement about this important variable

Changes in physical status

The intervention of cardiac operation seemed particularly beneficial in reducing the symptoms associated with physical exercise for the cyanotic child (Incapacity Table 5) Acyanotic children though initially less incapacitated showed less but also significant improvement Less striking but comparable changes in directions were noted in the child's level of physical activity as manifested in the testing situation This measure is based on the psychologist's assessment and is independent of the measure of incapacity rated by the physician When one compares the improvement within each group separately with changes in the combined non operated groups (1 + 2 + 35 + 45) it is only the cyanotic children who show significantly increased level of physical activity The normal children and cardiac children without surgery remain substantially unchanged

Change in maternal attitude

Improvement in the child was accompanied by significant changes in maternal attitudes The mothers of both cyanotic and acyanotic chil

Table 3 *IQ means for first and last tests and mean changes for Cyanotic group*
Two diagnostic categories

		First test		Last test		
Group (1)	*N* (2)	Mean (3)	S E (4)	Mean (5)	S E (6)	IQ change (7)
Cyanotic tetralogy of Fallot (A)						
Operated 4SA	21	97 9	3 5	104 0	3 5	+6 1
Nonoperated 4SA	18	93 9	5 3	99 5	4 6	+5 6
Other cyanotic conditions (B)						
Operated 4SB	12	103 5	3 9	107 2	5 3	+3 7
Nonoperated 4SB	27	95 1	3 7	92 1	4 1	−3 0

group the group was further subdivided into children with tetralogy of Fallot (Group 4 category A) and those with other cyanotic conditions (Group 4 category B) Table 3 shows the IQ means on the first and last tests in these diagnostic categories It was found that among non-operated children cyanotic tetrads improved in IQ while the other diagnostic category declined in IQ This difference in IQ changes between the two non operated subgroups was statistically significant The IQ changes found in the two diagnostic categories of operated cyanotic children were both positive (6 1 and 3 7) Almost no difference between operated and non operated cyanotic children was found in diagnostic category A (tetralogy of Fallot) the difference was large between operated and non operated children in category B (*cyanotic conditions other than* tetralogy of Fallot) Operated and non operated cyanotic tetrads gained in IQ while other cyanotic children gained only when operation intervened The difference in changes in IQ between the two diagnostic categories in the non operated but not in the operated group is understood only when one takes selective factors and disease severity into consideration Although age at testing arterial oxygen saturation hemoglobin and hematocrit were similar in all groups the non operated cyanotic group includes a larger number and a greater proportion of inoperable cases with an associated poor prognosis Thus one might expect greater differences between the two diagnostic cat gories in the non surgical group and small differences in the surgical group which a more nearly equal in terms of prognosis a initial condition There is a possibility that t decrease in IQ in the inoperable cyanotic ch dren may be related to altered attitudes physicians parents or testing psychologist

Emotional development

The greatest improvement in psychological a justment and behavioral variables is found the cyanotic group with surgery when o compares within group changes from the fi to the last test (Table 4) The reader is r minded that lower values of the variabl signify better adjustment less anxiety etc negative difference between the last and fi measurement represents improvement Amo all the groups it is only the operated cyano children who show statistically significant ga in general adjustment self confidence soc confidence willingness and attention An i teresting observation is that the non-operat cyanotic group showed a significant decline general adjustment Changes in this variab within the normal group and the two acyano groups remained unchanged Interveni surgery appeared to have little influence on t degree of physical dependence shown by t child

When one compares the improvement with each operated group separately with t

Table 6 *Maternal attitude*

Means at first and last tests and mean changes

Group (1)		N (2)	Mean at first test (3)	Mean at last test (4)	Difference (5)	s.e. of difference (6)	t (7)
(a) Protectiveness							
Well baby	1	36	11	2 22	11	12	93
Sibling	2	61	2 02	2 13	11	08	1 35
Acyanotic operated	35	34	3 68	2 88	− 80	21	−3 89[a]
Acyanotic nonoperated	35	46	3 37	3 07	− 30	13	2 38[a]
Cyanotic operated	45	33	4 15	3 24	− 90	21	−4 33[a]
Cyanotic nonoperated	45	48	4 23	4 10	− 13	13	− 96
(b) Pampering							
Well baby	1	37	2 54	2 54	0	17	0
Sibling	2	61	1 98	2 21	23	13	1 74
Acyanotic operated	35	34	3 50	2 68	− 82	20	−4 09[a]
Acyanotic nonoperated	35	46	2 67	2 89	22	16	1 39
Cyanotic operated	45	34	3 97	2 97	−1 00	23	−4 40[a]
Cyanotic nonoperated	45	48	3 94	3 71	−23	18	−1 27
(c) Maternal anxiety							
Acyanotic operated	35	33	3 97	3 06	− 91	30	−3 01[a]
Acyanotic nonoperated	35	46	3 50	3 37	− 13	13	− 99
Cyanotic operated	45	35	4 17	3 23	− 94	20	−4 69[a]
Cyanotic nonoperated	45	48	3 94	4 00	06	16	39

Negative change signifies improvement

[a] Mean changes from first to last test are significant using a single test level of 0 05

CONCLUSIONS

The effects of congenital heart disease on intellectual and emotional development have been studied Children with cyanotic and acyanotic cardiac disease their normal siblings and children selected from a well baby clinic were evaluated longitudinally over a five year period The effects of time and intervening cardiac operation on the children's development and in parental attitudes toward the child were measured

Cyanotic children showed a lag in intellectual development particularly in early years related in part to physical incapacity IQ increased in operated cyanotic children but not in the inoperable cyanotic group Children with acyanotic congenital cardiac disease were less incapacitated initially and their original IQ was closer to their true potential therefore less change was seen with time whether or not cardiac operation intervened

Greatest improvement in psychological adjustment and behavior was seen in the operated cyanotic children with less change in the other groups of normal children and those with heart disease After operation mothers of all cardiac children were less anxious about them and pampered and protected them less The data imply a relationship between the magnitude of parental concern and the presence of cyanosis a concern related only partly to the fact that these children were all more incapacitated

Physical ability increased in all children after operation but more so in those who were cyanotic preoperatively Physical status did not change in normal children and in unoperated children with heart disease

The data indicate that operative correction of the cardiac lesion and attendant alleviation of the accompanying symptoms are associated with improvement in intellectual functioning particularly in the cyanotic group In addition the visibility of cyanosis in particular appears to stimulate parental anxiety and its disappearance postoperatively is accompanied by the most marked decrease in parental concern

Table 5 *Physical status*
Means at first and last tests and Mean changes[a]

Group (1)		N (2)	Mean at first test (3)	Mean at last test (4)	Difference (5)	S E of difference (6)	t (7)
(a) *Physical level of activity*							
Well baby	1	37	3 62	3 67	05	15	32
Sibling	2	68	3 76	3 68	− 08	10	− 68
Acyanotic operated	3S	38	4 05	3 82	− 23	14	−1 61
Acyanotic nonoperated	3*S*	48	3 52	3 69	17	16	1 04
Cyanotic operated	4S	32	4 34	3 91	− 43	17	−2 41[b]
Cyanotic nonoperated	4*S*	49	3 88	4 14	26	19	1 36
(b) *Incapacity*							
Acyanotic operated	3S	36	2 08	1 58	− 50	24	−2 05[b]
Acyanotic nonoperated	3*S*	53	1 85	1 62	− 23	12	−1 80
Cyanotic operated	4S	39	4 46	2 77	−1 69	25	−6 79[b]
Cyanotic nonoperated	4*S*	46	4 00	4 04	04	16	27

[a] Negative change signifies improvement
[b] Mean changes from first to last test are significant using a single test level of 0 05

dren who had surgery became less anxious and pampered and protected their children less when compared with the mothers of children who did not have surgery (Table 6) The changes were statistically significant It must also be noted that the mothers of acyanotic children who did not have surgery also became less protective during the period from the first to the last test The significant change in this group appears to be related to the decline in symptoms in this group with age and with a possibly greater assurance by the mother of the acyanotic child who does not look as sick as the blue child

Comparison with earlier studies

There are a limited number of longitudinal studies of development in children with congenital heart disease comparing preoperative and postoperative status Although Landtman (4) found relatively normal intelligence in his group of children with congenital heart disease 72% had an increase in IQ following operation while 28% showed a decrease An increase of over 10 IQ points occurred in 23 postoperatively while an equivalent decrease was noted in only four Brett & Kohler (2) on the other hand found a higher incidence of lower IQ in children with cyanotic congenital heart disease and this group showed a post operative increase in intelligence quotient Malaspina (8) recorded a slight increase in the intelligence quotient in a small group of children with congenital heart disease tested 6 to 24 months after operation

In a qualitative evaluation of behavioral changes Landtman found less preoperative deviation from the normal and less postoperative change These differences may be related to population variation or differences in testing procedures Still, he described the over all behavior of 56 of 84 children as improved after operation

Of a small group of 25 over protective mothers (4) 11 became less over protective after successful operation This change is not as marked as in our study but does not differentiate between parental attitude of parents with cyanotic and acyanotic children

In a recent study Drash & Money (3) were concerned with the decrease in postoperative IQ in 2 of 4 children but this concern is not borne out in studies of larger numbers However the possibility is not eliminated that transient IQ deficit may be present in the early postoperative period which is no longer evident six months after operation when re evaluation was done in our study

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BLADDER STONE DISEASE OF CHILDHOOD

I *An epidemiological study*

A L AURORA O P TANEJA and D N GUPTA

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Calculus disease of the urinary tract has probably been known since antiquity The most ancient evidence of this comes from Egyptian mummies (20) Ruffer described three vesical calculi found by Flinders Petrie in a Predynastic skeleton (20) In 1901 Elliot Smith discovered a calculus lying among the pelvic bones of the skeleton of a boy aged 15 years whose grave was exhumed at El Amarah in upper Egypt (11) Urinary calculi (asmari) seem to have been fairly common in India (4 10 12) It was considered a dangerous ailment comparable to the God of death The condition has been also described by Hippocrates (15) Celsus (24) Galen (11 24) Albucasis (15 24) and Morgagni (1) Once a common entity in Western Countries (13 14 21) it has practically disappeared from those countries presumably due to the rise in living standards It continues to be a pediatric problem in the developing countries of South East Asia (2 3 5 16 17 23) where poverty and poor hygiene still prevail

The relationship of bladder stone disease of childhood to the more universally distributed renal calculi has not been clearly defined The etiology and pathogenesis of this condition also remain obscure It was with this background that the present studies were undertaken

MATERIAL AND METHODS

Records of all proved cases of urinary calculi admitted to Safdarjang and Irwin Hospitals New Delhi during the years 1963 and 1964 were studied A case was considered proved if he/she had spontaneously passed the stone(s) in the hospital or the stone(s) was/were removed surgically or/and radiological examination including intravenous pyelography clearly showed the presence of stone(s) All such cases were analysed as to the location of stone and age and sex of the patient

The incidence of urolithiasis in relation to total surgical admissions and total attendance to the out patient departments of the hospitals was recorded

It may be pointed out that these two hospitals chiefly serve the poor and lower middle classes of the society of Delhi and its neighbouring areas

Hospital records of Sen's Nursing Home New Delhi a large private Nursing Home catering to the needs of higher middle class and rich persons were similarly analysed for the years under study

Forty four cases of bladder stone in children were followed (by questionnaire) for a period of 1 ? to 9) years for any recurrence

Heights and weights of children in the age group 3 to 14 years admitted to two schools in Delhi were recorded and compared with the heights and weights of bladder stone cases to assess deviation if any in the growth patterns in these three groups Children of one school belonged to poor families and those of other school came from rich families

Meteorological data was obtained from the Director Meteorological Department of India and seasonal incidence of disease was recorded In making this analysis the time of occurrence of the disease was

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REFERENCES

1 Anastasi A Standardized ability testing *in* P H Mussen (ed) *Handbook of Research Methods in Child Development* John Wiley & Sons New York 1960 p 456
2 Brett J & Kohler C Incidences neuropsychiatriques des cardiopathies congénitales chez l enfant *Pediatrie 11* 59 1956
3 Drash P W & Money J Statural and intellectual growth in congenital heart disease in growth hormone deficiency and in sibling controls *in* D B Cheek (ed) *Human Growth Body Composition Cell Growth Energy and Intelligence* Lea and Febiger Philadelphia 1968 p 606
4 Landtman B Valanne E Pentti R & Aukee M Psychosomatic behavior of children with congenital heart disease Pre and postoperative studies of eighty four cases *Ann Paediat Fenn* 6 1 1960
5 Linde L M Rasof B Dunn O J & Rabb E Attitudinal factors in congenital heart disease *Pediatrics 38* 92 1966
6 Linde L M Rasof B & Dunn O J Mental development in congenital heart disease *J Pediat 71* 198 1967
7 Linde L M Dunn O J Schireson R & Rasof B Growth in children with congenital heart disease *J Pediat 70* 413 1967
8 Malaspina M & Bono G Considerazioni sullo sviluppo mentale dei cardiopatici congeniti *Minerva Cardioangiol 3* 207 1955
9 Rasof B Linde L M & Dunn O J Intellectual development in children with congenital heart disease *Child Develop 38* 1043 1967

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Table 1 *Prevalence of urolithiasis in the two sexes*

Population studied (1963–64)	Kidney stone (All ages) M F	Kidney stone Children 0–14 years M F	Bladder stone (All ages) M F	Bladder stone Children 0–14 years M F
Poor population TA – 182040 M F – 1 2 1 SA – 44273 M F – 1 7 1	6 1 1	6 3 1	28 6 1	33 6 1
Well to do population TA – 1538 M F – 2 4 1 SA – 985 M F – 2 5 1	2 7 1	Male 0 Female 1 case	Male 3 cases Female 0	Male 0 Female 0

TA – Total admissions
SA – Surgical admissions
M – Male
F – Female

calculated from the date of commencement of symptoms clearly attributable to urolithiasis rather than the date of presenting at the hospital

Sixty one cases of bladder stone disease in children were analysed as to the urban and rural distribution Any relevant family or past history was recorded in these cases

The data on hardness of water supplied to the population of Delhi and New Delhi was obtained from the Chief Water Analyst Delhi

RESULTS

A total of 1 952 774 patients attended the out patient Departments of Irwin and Safdarjung Hospitals The total number of admissions were 182 040 and of these 44 273 were admitted to the surgical wards There were 396 cases of bladder stone and 233 cases of renal lithiasis

In Sen s Nursing Home 1984 patients attended as out patients The total number of admissions was 1538 and of these 985 were admitted to the surgical wards There were 3 cases of bladder stone and 18 cases of renal lithiasis

The prevalence of urolithiasis in the two groups of population in the two sexes is given in Table 1 It is obvious that both renal and bladder calculi tend to be more frequent in males of the poor population than in the well to do Further the bladder stone cases are four to five times more frequent than kidney stones in the poor population Bladder stones are rare in the well to do

The incidence of calculi in various age groups is given in Fig 1 It will be seen that in the poor population there are two peaks for bladder stone cases one in the age group 0–10 years and the other after 45 years The kidney stone cases show a gradual rise up to the age 20–24 years after which there is a gradual fall to be followed by another rise after the age of 45 years The rise in both kidney and bladder stone cases after 45 years could possibly be due to obstructive uropathies In the well to-do there was no case of bladder stone in the 0–14 years age group and there were only 3 cases in the later ages The kidney stone cases showed a trend very similar to those in the poor population

The incidence of urolithiasis expressed as a percentage of surgical admissions in both sexes of the two population groups is shown in Fig 2 It will be observed that in the poor population bladder stone cases in children are six times more frequent than kidney stones whereas bladder stone cases for all ages are nearly 1 ¹/ times more frequent than the kid

ney stones This situation is reversed in the well to-do population where kidney stones were six times that of the bladder stones Further it should be stated that the incidence of urolithiasis in the well to-do population is higher than in the poor population when the data are expressed as percentages of the total and surgical admissions as given below

Population	Percentage of total admissions	Percentage of surgical admissions
Poor	0 43	1.58
Well to-do	2 27	3 54

The above epidemiological data regarding the prevalence of the disease clearly shows that bladder stone cases are more frequent in the poor population especially in the male children whereas the kidney stone cases are often observed in the well to-do Urolithiasis in children is a rarity in the latter class of population

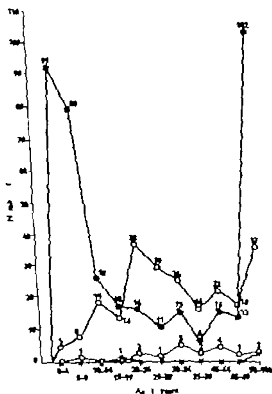


Fig 1 Incidence of urolithiasis in various age groups
Bladder stone cases ●—● poor population 396 cases — well to-do population 3 cases. Kidney stone cases o—o poor population 233 cases o—o well to-do population 18 cases

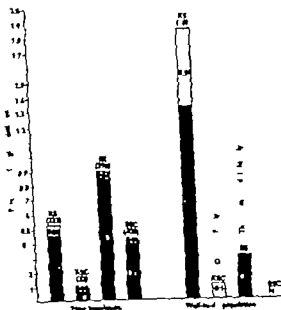


Fig 2 Incidence of urolithiasis expressed as percentage of surgical admissions (SA)
Male ■ Female □ Poor population total SA = 44,273 M F = 1 7 1 Well to-do population total SA = 985 M F = 2 5 1 *KS* = Kidney stone *KSC* = kidney stone in children *BS* = bladder stone *BSC* = bladder stone in children Values in parentheses indicate the total no of cases

Recurrence in bladder stone cases after surgical removal

Since 1955 44 cases of bladder stone disease who underwent surgery have been followed for a period varying from 1 $^1/_2$ to 9 $^1/_2$ years as shown in Table 2 It is significant to note that there was not even a single case with persistence or recurrence of symptoms of bladder stone disease This shows that following surgical removal if recurrence does take place in

Table 2 Follow up of 44 cases of bladder stone in children

Duration of follow up (years)	No of cases	Recurrence
1½ to 3½	20	NIL
5 to 7½	9	NIL
8 to 9½	15	NIL

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Table 4 *Seasonal incidence of cases with bladder stone in children*

Months	Present series 61 cases (1962–65) No of cases	%age of 61	Total cases studied 147 (1955–65) No of cases	%age of 147
January	5		13	
February	2	19 7	9	25.2
March	5		15	
April	4		9	
May	3	22 9	9	24 5
June	7		18	
July	3		9	
August	5	32.8	17	29 9
September	12		18	
October	4		9	
November	9	24 6	14	20 4
December	2		7	

Family and past history

The size of the family varied from three to six members All 61 cases belonged to poor or lower middle class Some 80 3 came from very poor families with an income of less than 150 rupees per month and 19 7 belonged to the lower middle class group with an income ranging from 151 to 400 rupees per month

In none of these cases was a family history of bladder stone available The father of one case and an uncle of another case gave histories of passage of stone per urethrum after attacks of renal colic Past history of diarrhoea or infectious fever prior to the onset of symptoms of bladder stone disease was available in six patients Two patients had episodes of diarrhoea 6 months before the onset of bladder stone disease In two patients a history of smallpox 7 months and 10 months prior to the onset of symptoms of bladder stone was available Two patients suffered from measles immediately before they had the symptoms attributable to bladder stone

Hardness of water

The water supplied to the residents of Delhi and New Delhi has a hardness ranging from about 120 to 225 ppm (expressed as calcium carbonate) This degree of hardness is well below the maximum total hardness of 600 ppm permitted for drinking water

DISCUSSION

(a) Prevalence

A high prevalence of bladder stone cases in contrast to renal calculi has been reported from Thailand (5 6 17 23) South China (19) and India (2 3 22) In these studies the method of investigation has been either a population survey or an analysis of the hospital records Passmore (17) Sanong Unakul (23) and Halstead & Valyasevi (6 7) have reported high prevalence of bladder stone disease in north-east Thailand on the basis of population surveys Sanong Unakul (23) in his extensive statistical survey of the problem of urolithiasis in Thailand reported an incidence of 0 to 10 29 per 10 000 for the entire country In this series vesical calculi constituted 82 0 of all stones Passmore (17) quoting Dr Cholvit Chutikorn Hospital Director of Ubol Provincial Hospital states that in a period of 7 years whereas 1250 vesical calculi had been removed there were only one or two renal calculi each year In his analysis of hospital records of Canton hospital in South China Thomson as quoted by Siddall (19) could find 2962 cases of vesical calculi as compared to only 5 cases of renal stone

The prevalence of bladder stone disease in various parts of India has been highlighted by McCarrison (16) Rao (18) and Andersen *et al* (2) The figures given by McCarrison (16) for whole of India range from 0 to 43 8 per 10 000 population Andersen *et al* (2) reporting on the bladder stone cases from Ahmadnagar (1953–54) found an incidence of 0 85 per 10 000 population The latter workers also studied 61 children with bladder stone from 1951–57 During this period 4452 children were admitted to the hospital giving an incidence of 1 3 of all pediatric admissions Aurora & Ramalingaswami (unpublished data) collected figures for urolithiasis from various

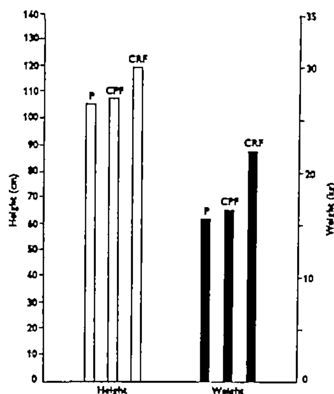


Fig 3 Growth pattern in cases of bladder stone disease and in control children Age group 5–9 years *P* = Bladder stone cases *CPF* = children of poor families *CRF* = children of rich families

bladder stone cases of childhood it must be very rare

Growth patterns in cases of bladder stone disease

This was assessed on the basis of heights and weights recorded in some of the patients The data have been compared with school children belonging to poor and rich families, who did not have any clinical evidence of bladder stone disease The results are given in Fig 3 It will be noted that the growth patterns of the bladder stone cases and of the children from poor families are essentially similar On the other hand children from rich families had statistically better growth patterns than both the bladder stone cases and children from poor families It is thus apparent that the growth pattern of bladder stone cases is in no way different from the generally poor population from which most of the cases come

Seasonal prevalence of bladder stone cases

The meteorological data regarding the temperature, rainfall and relative humidity for the years 1962–1965 are given in a consolidated form in Table 3 and the incidence of bladder stone cases is recorded in Table 4

It will be seen that there is no significant difference between the number of cases in summer and winter while many other cases had their first symptoms in the rainy season

Urban and rural distribution of bladder stone cases

In 61 cases of bladder stone in childhood 52 5 % of the cases belonged to the urban population while 47 5 % came from rural population

Table 3

Months	Temperature (C) Maximum	Temperature (C) Minimum	Rainfall (mm)	Relative humidity ( )
January	19 2 to 22 6	4 5 to 8 6	0 0 to 33 2	75 to 80
February	23 8 to 26 5	9 1 to 11 5	1 5 to 12 6	67 to 77
March	29 3 to 32 1	14 0 to 16 2	1 0 to 8 0	45 to 60
April	33 3 to 37 0	19 3 to 22 5	0 0 to 13 9	38 to 50
May	38 4 to 40 8	23 5 to 28 3	0 0 to 16 0	35 to 41
June	38 4 to 40 8	27 1 to 28 7	3 0 to 117 6	38 to 57
July	33 0 to 37 6	24 5 to 27 6	45 8 to 538 2	68 to 83
August	32 4 to 34 4	24 9 to 26 0	84 6 to 446 3	72 to 87
September	32 3 to 34 2	22 9 to 23 7	181 5 to 277 7	66 to 79
October	32 7 to 33 6	16 9 to 20 1	0 0 to 0 2	57 to 63
November	27 7 to 29 8	10 6 to 14 9	0 0 to 2 0	59 to 68
December	21 6 to 23 2	6 1 to 7 8	0 1 to 27 4	68 to 82

low the a e of 14 years and again after 50 years In the old age groups the stones are nearly always secondary to obstructive uropathies

The overall prevalence of urinary stones in males is high The occurrence of bladder stone cases as compared with kidney stone cases in males is also distinctly more frequent In the present series in the poor population the male to female ratio was high especially in case of bladder calculi

The higher incidence of bladder stone cases in males has been explained on the basis of the anatomical difficulty for the escape of stone presented by the male bladder neck and by the long tortuous urethra In order to test the validity of this hypothesis zinc spheres 1 8 cm or 1 2 cm in diameter were introduced in the bladders of male rabbits Of the four spheres introduced three were expelled and one was retained The two smaller spheres of 1 2 cm were expelled in 3 to 4 days and of the two larger spheres one was expelled in 42 days It may be mentioned that the larger sphere which was retained was heavier than the similar sized sphere which was expelled This becomes of considerable significance when it is realized that human bladder has much greater musculature and the male urethra in humans is certainly wider than the rabbit urethra Furthermore the zinc spheres used in the animal experiments were much larger than many of the stones recovered from the bladders of children Thus a long narrow urethra in the male *per se does not explain the higher frequency* of bladder stone in this sex Again it is not only in the bladder but also in the case of kidney stones that the prevalence in males is higher than in females For the retention of stones in the urinary tract it is probable that the stones in early stages stick to the urothelium so as not to be expelled by the stream of urine or contractions of the bladder The explanation for the higher prevalence in males may be the possible role of sex hormones deficiency of some calculus inhibitor in the urine and role if any of the prostate gland

SUMMARY

An epidemiological study of cases of urolithiasis in poor population and affluent society was undertaken The study revealed a great preponderance of bladder stone cases in childhood in the poor population as against its marked paucity in the well to-do The condition occurred predominantly in the male children The follow up studies show that bladder stones in children do not recur after surgical removal This study fails to show any seasonal relationship in the prevalence of this malady In the present series there was no family history of bladder stone and the cases were nearly equally distributed among the urban and rural population The findings of the present study are discussed in the light of the pertinent literature

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REFERENCES

1 Alexander B *The seats and causes of diseases investigated by anatomy* Hafner Publishing Co New York 1960 Vol II Letter XLII
2 Andersen D A Sriramachari S & Khandagale M K Investigations into relationship between bladder stones and malnutrition *Indian J Med Sci 17* 617 1963
3 Aurora A L Ramalingaswami V & Gaitonde P D Bladder stone disease in children in Delhi area *J Urol 91* 347 1964
4 Castiglioni A *A history of medicine* Translated from the Italian and edited by E B Krumbhaar Routledge & Kegan Paul London 1947 p 97
5 Halstead S B Bladder stone in Thailand A review of the problem *Amer J Trop Med 10* 918 1961
6 Halstead S B & Valyasevi A *Clinical and epidemiological aspects of vesical calculus disease in Thailand and elsewhere In* compilation of papers presented at a workshop on clinical and experimental urolithiasis held at the Naval *Medical Research Institute* National Naval Medical Center Bethesda Maryland on 8 and 9 June 1965
7 — Studies of bladder stone disease in Thailand I Introduction and description of area studied *Amer J Clin Nutr 20* 1312 1967
8 — Studies of bladder stone disease in Thailand

hospitals in India High incidence of urolithiasis was reported from Moradabad district, where a visit showed the abundance of renal lithiathis and only few cases of bladder stone in children This is in contrast to high prevalence of bladder stone cases in some areas of Thailand

The epidemiological data collected from three large hospitals in the present series shows that bladder stone cases constitute nearly half of all cases of urolithiasis and of these nearly 50% occur in children (0–14 years) The data from Safdarjung and Irwin Hospitals which cater for the poor population show 47 3% of bladder stone cases in children compared with 12 47% of kidney stone cases The incidence of bladder stone and kidney stone cases expressed as a percentage of the pediatric surgical admissions was 1 62% and 0 26% respectively On the other hand the data from Sen s Nursing Home hospital which serves the affluent society revealed that whereas no case of bladder stone occurred in children (0–14 years) one case of kidney stone was observed in this age group Thus in children of privileged class urolithiasis is rare This trend is similar to the observations made in the developed nations of the world once a common malady in the children of Western countries it is now a rarity with the rise in living standards The paucity of bladder stones in children in areas where renal lithiasis abounds and vice versa suggests that the factors operating in renal lithiasis are not directly concerned in the genesis of primary bladder stone disease of childhood

(b) Rural and urban prevalence

Passmore (17) and later Halstead (5) and Halstead & Valyasevi (8) have indicated the rarity of bladder stone cases in urban areas and their high prevalence among the rural population of Ubol Province (Thailand)

In the present series though no comparable population surveys were conducted the data strongly suggest that the prevalence of bladder stone cases in urban and that in rural population are similar In this study 52 5% of 6 cases belonged to the urban population while 47 5% came from rural areas It is probable that the children of the poor urban population who chiefly suffer from this disease live under conditions similar to those of rural population

(c) Climatic factors

Dehydration consequent on excessive sweating in summer leading to the concentration of urine and thus disturbing the crystalloid colloid ratio has been implicated in the genesis of stone (9) The study of 61 cases of present series and 147 cases over the last 10 years fails to show greater incidence of the disease in summer In the evaluation of this data the time of occurrence of the disease was calculated from the date of commencement of symptoms clearly attributable to urolithiasis rather than the date of presenting at the hospital It would have been observed that there is slightly greater incidence of disease during rainy season due to rather more cases in September in the present series It might be argued that symptoms appear when the stone has attained a certain size and the duration of symptoms may not necessarily indicate the time of initiation of the stone Thus dehydration might be implicated indirectly as a factor in the genesis of stone If this be so then there is no reason why recurrence should not be frequent in the patients who have once formed stone

(d) Family history

Halstead & Valyasevi (6) in their studies from Thailand state that the persons who shared the same food and household environment as the stone cases were twice as likely to develop stones as the average In the present series in none of the cases was a family history of bladder stone available

(e) Age and sex

The data in the present series are comparable to the series reported by other workers (2, 22) Maximum numbers of cases are observed be

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CONGENITAL HYPOPLASTIC THROMBOCYTOPENIA AND CEREBRAL MALFORMATIONS IN TWO BROTHERS

HANS M. HØYERAAL, JON LAMVIK and PETER JOHAN MOE

From the Department of Paediatrics and the Gade Institute, Department of Pathology, University of Bergen, Norway

At least two syndromes comprising disturbed blood cell production and congenital abnormalities have been reported.

In 1927 Fanconi (7) described three brothers 5–7 years of age who developed chronic pancytopenia which proved fatal within a year of onset. Patients with Fanconi syndrome often have multiple congenital anomalies: abnormal pigmentation of the skin, stunted growth, strabismus, exaggerated reflexes and microcephaly. Aplasia of the radius is only reported in a few cases (3, 11).

Isolated congenital hypoplastic thrombocytopenia associated with one or more anomalies has been reported in at least 35 cases (4, 5, 8, 12, 15, 18) since Greenwald & Sherman in 1929 reported the first case (9). Cases with known intrauterine viral infections have been excluded. Aplasia of the radii seems to occur in about [illegible] of the cases. In fact an attempt has been made to separate a special syndrome consisting of aplasia of the radii, amegakaryocytic thrombocytopenia and leukemoid reaction from the other cases of congenital hypoplastic thrombocytopenia (1, 6, 15). The most frequent other abnormalities are other skeletal defects, cardiac and urogenital anomalies.

Only one case of persistent congenital hypoplastic thrombocytopenia associated with microcephaly has been found in the literature (5).

The purpose of the present report is to describe the clinical, hematological and pathological findings in two brothers with congenital hypoplastic thrombocytopenia, microcephaly with cerebellar atrophy and a probable hemolytic anemia.

CASE REPORTS

Case 1. S. M., a male infant born on the 4th September 1958 following an uneventful pregnancy of 41 weeks. The amniotic fluid was foul-smelling and green. Birth weight was 2400 g and length 45 cm. He was asphyxiated but cried after 12 min following resuscitation. At the age of three months he was admitted to the Children's Hospital, Bergen, because of failure to thrive. He was microcephalic (largest head circumference of 35.5 cm, 2 cm below the 2 [illegible] percentile for age) and had lateral squinting of the eyes. Motor activity was decreased whereas muscular tone was increased. No reflex abnormality was elicited. Routine blood investigations and EEG revealed no abnormalities. Roentgenograms of the skull showed prominence of the sagittal suture. At that time no definite diagnosis was made.

At the age of 4 months he had a left-sided pes equinovarus corrected with plaster of Paris. He bruised readily from the age of 9 months. Psychomotor development was retarded.

On his second admission at the age of 21 months he gave the impression of mental retardation. His length, 75 cm, was 3 cm below the 2 [illegible] percentile for age, his weight 7.7 kg was 0.7 kg below the 2 [illegible] percentile for length, whereas his head circumference 41 cm was 4.7 cm below the 2 [illegible] percentile for age. He was spastic, particularly in the lower extremities. Multiple ecchymoses and petechiae were seen. The platelet count using [illegible]'s method (13) varied between 13 000 and 41 000 per mm. The bleeding time (finger puncture) was markedly prolonged, the

III Epidemiological studies in Ubol Province *Amer J Clin Nutr 20* 1329 1967
9 Harlin H C A logical approach to the problem of urinary calculi prophylaxis *New York State J Med 55* 213 1955
10 Joly J *Indian Medicine* C G Kashikar (Tilak) Poona (India) 1951 pp 123–125
11 Joly J S The etiology of stone *J Urol 32* 541 1934
12 Kutumbiah P *Ancient Indian Medicine* Orient Longmans Calcutta 1962 p 167–168
13 Lett H On urinary calculus with special reference to stone in the bladder *Brit J Urol 8* 205 1936
14 Lowsley O S & Kirwin T J *Clinical Urology* Williams & Wilkins Baltimore 1940 Vol II pp 1025–6
15 Mettler C C *History of Medicine* Blakiston Philadelphia 1947 p 696
16 McCarrison R A lecture on the causation of stone in India *Brit Med J I* 1009 1931
17 Passmore R Observations on the epidemiology of stone in the bladder in Thailand *Lancet I* 638 1953
18 Rao M V R *Report on the studies on vesical calculus (stone in the urinary bladder) in Mehsana District (1953–55)* Director Government Printing Publications and Stationery Bombay State Bombay 1958
19 Siddall A C Primary vesical calculus *J Urol 37* 268 1937
20 Sigerist H E *A history of Medicine* (Vol I Primitive and Archaic Medicine) Oxford University Press New York 1955 p 63
21 Thomas J M R Vesical calculus in Norfolk *Brit J Urol 21* 20 1949
22 Tiwari C P Bladder stones in children *Indian Practitioner 11* 1055 1958
23 Unakul S Urinary Stones in Thailand A statistical survey *Siriraj Hospital Gazette 13* 199 1961
24 Winsbury White H P *Stone in the urinary tract* Butterworth London 1954 2nd edition

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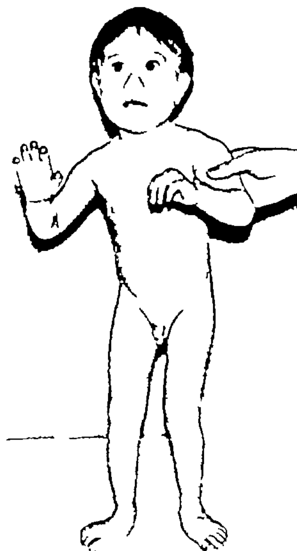


Fig 1 Photograph of case 2 at the age of 20 months

coagulation and prothrombin times were normal There was no anemia or leucopenia but relative granulocytosis The erythrocyte sedimentation rate ranged from 41 to 72 mm per hour Occult blood was found on repeated stool examinations Bone marrow smears were reported to show abundant megakaryocytes He died almost 23 months old 36 hours after a head injury Prior to death peripheral blood smears revealed 23 nucleated red blood cells per 100 white blood cells

Autopsy revealed a subdural hematoma The brain was small 610 g with a very small cerebellum weighing only 1 5 per cent of the whole brain The lateral cerebral ventricles were dilated particularly in the posterior part The vermis as well as the cerebellar hemispheres were hypoplastic with a thin cortex *Microscopically there was atrophy of the granular* layer while the Purkinje cells were present in normal number many appeared to be displaced into the molecular layer The dentate nucleus showed no signs of atrophy Bone marrow sections from femur and columna showed a differentiated cell picture however megakaryocytes were scanty

Case 2 N O M a younger brother of case 1 was born on 26th April 1965 In 1960 his mother had an abortion in the 4th month Her uterus contained several myomas In the present gestation of 40 weeks she was given regular norethisterone (Primolut N) *injections from the 2nd to the 7th month* but no thiazide therapy The amniotic fluid was yellow Birth weight was 2200 g and length 45 cm The delivery and neonatal period were uneventful Psychomotor development was retarded He was admitted to the Children's Hospital as his mother felt that he might have the same disease as his brother He had readily bruised from the age of 5 months and was very small

On admission at the age of 19 months his length 74 5 cm corresponded to the 2 / percentile for age and his weight 7 6 kg was / kg below the 2 percentile for length He was pale microcephalic spastic and had a similar peculiar appearance (Fig 1) to his brother The largest head circumference was 42 cm 3 cm below the 2 / percentile for age A cerebellar type of ataxia was also noted He had several purpuric lesions of the skin and mucous membranes but no lymphadenopathy or hepatosplenomegaly

Laboratory investigations

His haemoglobin concentration was 8 g per 100 ml The platelet count using Kristenson's method (13) ranged from 42 000 to 16 000 per mm³ The bleeding time (finger puncture) was markedly prolonged whereas the coagulation and prothrombin times and Hess's test were normal Few megakaryocytes were seen on repeated bone marrow smears These were both large and small mostly immature forms

Roentgenograms of the skeleton showed retarded bone development Air-encephalograms showed marked dilatation of the lateral ventricles cortical atrophy and increased width of the *septum pellucidum* most probable caused by a noncommunicating cyst

Amino acid chromatography revealed increased urinary histidine (and alanine) excretion and low serum valine leucine and isoleucine concentration (Dr S Halvorsen Pediatric Research Institute Rikshospitalet University of Oslo and Dr L Gjessing Dikemark Hospital Asker) Chromosome studies showed a normal male karyotype (46 xy) Thrombocyte antibodies (complete and complement fixing) were not found (Dr Kissmeyer Nielsen Immunohematological Department Kommunehospitalet Arhus) *Platelet* survival studies with transfused Cr labelled platelets showed a normal life span of 8 9 days (Dr A Foss Abrahamsen Oslo City Hospital and Dr K *Halvor*sen Immunohematological Department University of *Bergen)*

Periodically there were signs indicating a *hemolytic* process with a sudden fall in hemoglobin concentration and an increase in the number of reticulocytes

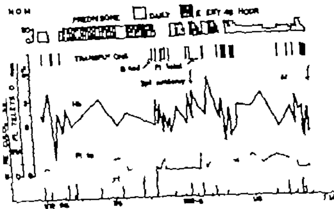


Fig 2 Case 2 Course from the age of 19 months to 3 / years showing therapy and main hematological data

Nucleated red cells were also found in peripheral blood smears He was slightly icteric several times serum bilirubin was 1 3 mg per 100 ml Osmotic fragility curves only once revealed slightly increased fragility Spherocytosis was not seen Direct Coombs's test remained negative Serum values of vitamin B and folic acid were normal Serum haptoglobin was 70-80 mg per 100 ml Hemoglobin moved in the A and A fractions during disc electrophoresis

Enzymatic studies of the red blood cells at 25 C and pH 7 5 showed normal activity for the following enzymes hexokinase phosphofructokinase phosphofructoaldolase pyruvic kinase lactate dehydrogenase glucose 6 phosphate dehydrogenase and glutathion reductase Adenine nucleotides were also normal These studies were carried out by Dr M Hjelm The Academic Hospital Uppsala

Erythrocyte survival studies with ^{51}Cr labelled cells showed half life time of 13 days of the patient's erythrocytes in a normal recipient (Dr K Halvorsen Immunohematological Department, University of Bergen) Scanning of liver spleen and bone marrow following autotransfusion of ^{51}Cr labelled red blood cells suggested the spleen as the main destruction site (Dr K E Egeberg Clinical Physiological Department University of Bergen)

The age and course

The patient received prednisone therapy most of the time for years without definite effect on the thrombocytopenia Nearly half the observation time he was on prednisone every other day (Fig 2) Repeated courses of supplementary therapy with anabolic steroids had no demonstrable clinical or hematological effect Prednisone was maintained mainly because of possible beneficial effect on the clinical condition bleeding tendency and the hemolytic process Because of hematemesis and melena he periodically required blood transfusions (Fig 2) Five platelet transfusions gave only short lasting effect on the platelet counts

During his last) year of life he suffered from recurrent otitis gastroenteritis and aphtous stomatitis Increasing brown skin pigmentation was seen

Four months after having had chickenpox he had herpes zoster Gamma globulin quantitation by radial diffusion technique in agar revealed a hypo IgG (450-550 mg per 100 ml) IgM was slightly increased (Dr O Tønder Microbiological Department University of Bergen) Test for antibody to toxoplasma in indirect hemagglutination gave pathological titre (160) but the Dye test was less than 4 Roentgenograms of the skull revealed no calcification and no evidence of toxoplasmosis was found on examination of the eye Splenectomy was performed in December 1967 in an attempt to control the hemolytic process and possibly influence the thrombocytopenia The weight of the spleen was 55 g Histologically the spleen showed hyperemia No pigment deposits were seen During the last months of life the patient had recurrent febrile periods He received several courses of antibiotic therapy except terminally H died at the age of 3 / years

Autopsy revealed a small brain 613 g against the normal weight of 1150 g The macroscopical appearance was almost identical to that found in case 1 The cerebellum was hypoplastic weighing only / of the normal weight with small symmetrical hemispheres and vermis There was cerebral oedema with thickened meninges which showed microscopical signs of subacute inflammation The posterior parts of the lateral cerebral ventricles were dilated, most marked on the right side A septum pellucidum cyst (Fig 3) was found with an anteroposterior diameter corresponding to the whole length of corpus callosum, which was quite thin while the anterior commissure and the fornices appeared normal

No definite microscopical abnormality was noticed in the cerebrum The cerebellar cortex was thin with an atrophic granular layer (Fig 4) The Purkinje cells were numerous many appeared displaced into the molecular layer Some showed club-like expansions of th dendrites The dentate nuclei and the inferior olives appeared normal

The thymus was small and to a great extent replaced by fat There was a lack of cortico-medullary differentiation The lobes were composed of spindle formed cells with scattered Hassal's corpuscles

There was hemorrhages into the skin gut endocar

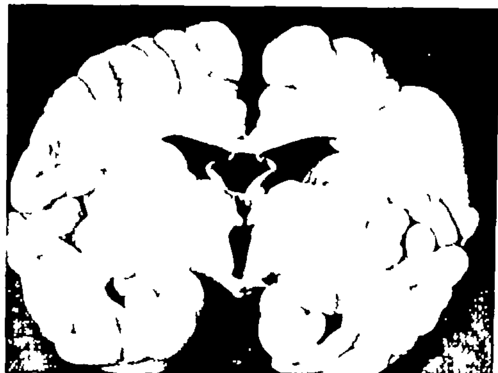


Fig 3 Case 2 Front section of the cerebrum showing the cystic cav in the septum pellucidu and the dilated lateral v tricles

dium left kidney and pelvis and the bone marrow was hypoplastic with very few megakaryocytes

The post mortem examination in addition revealed giant cell pneumonia centro lobular hepatic degeneration moderate renal tubular degeneration and adiponecrosis around the pancreas

Family studies

The parents of the reported siblings an older brother and a sister born in 1967 were all healthy Clinical hematological and marker gene studies were performed in 26 members of the family Five of them gave a history of bruising readily but only one platelet counts (142 000–104 000 per 100 mm³) low normal range One had congenital defect a palate This of course could just as well have curred in a random population

The younger sister of the reported cases and other members of the family had IgG below 800 per 100 ml (450–750 mg per 100 ml)

Owing to the positive hemagglutination test in 2 all the 26 members of the family were tested antibodies to toxoplasma The mother and three cousins had a positive hemagglutination and Dye

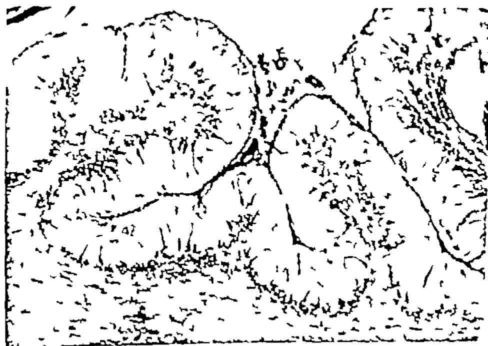


Fig 4 Case 2 Microscopic section of the cerebellar hemisphere showing atrophy of the granular layer and prominent Purkinje cells Htx+E ×60

The youngest sister like case 2 had a positive hemagglutination test, but a negative Dye test. Two other members of the family had questionable positive tests.

No abnormality or inconsistency was found of the ABO Rh MN Kell groups (Dr C Bruun Hansen Immunohematological Department University of Bergen) Gc and Hp types (Dr Reinskou Department of Forensic Medicine University of Oslo) Gm typing (Dr J B Natvig Rheumatological Research Department University of Oslo) and PGM typing (Dr E Monn Department of Forensic Medicine University of Oslo)

DISCUSSION

The reported siblings were very similar. They were both small for dates. They bruised readily from the age of a few months had chronic thrombocytopenia microcephaly retarded growth mental retardation a peculiar appearance and spasticity. Autopsy revealed a small brain and a hypoplastic cerebellum in both. A cerebellar atrophy of the granular layer type (14) was evident suggesting destruction or lack of formation of the afferent neurons.

The normal survival of homologous platelets and lack of antibodies suggest thrombocytopenia due to insufficient production. The few megakaryocytes in the bone marrow smears from case 2 appear to be compatible with this assumption. Case 1 had abundant megakaryocytes in bone marrow smears when alive, scanty in bone marrow sections at autopsy. These findings in two siblings suggest that there is actually very little if any difference between cases of hypoplastic thrombocytopenia and the cases of amegakaryocytic thrombocytopenia. The same impression is gained from the literature (5 10 12 18 21)

Even the morphology of the megakaryocytes may be an unreliable criterion of lack of platelet production. Myllylä *et al* (10) for instance demonstrated normal number of megakaryocytes without any clear morphological abnormality in 2 of their 15 cases of "hereditary thrombocytopenia" considered to be due to a defect in platelet production.

These two siblings seem to be the first reported cases of congenital hypoplastic thrombocytopenia associated with hypoplasia of the cerebrum and cerebellum. One may speculate as to whether this represents a new syndrome or if it is just a variant of the previously reported cases of hypoplastic thrombocytopenia. Eisenstein published in 1966 (5) a prematurely born infant with congenital amegakaryocytic thrombocytopenic purpura. Psychomotor development appeared normal, yet he was microcephalic, had a short stature and was unquestionable spastic. He had no ecchymoses or petechiae when last seen at the age of 22 months and his platelet count was at that time 80 000 per mm³

No skeletal defects were found in the reported siblings and some of the clinical features (microcephaly retarded growth and mental development exaggerated deep tendon reflexes and pigmentation) are more in common with Fanconi anemia than with hypoplastic thrombocytopenia. Cerebral hypoplasia associated with severe hypoplasia of the cerebellum does not seem to have been reported in Fanconi syndrome either. No signs of erythropoietic hypoplasia were seen in the reported siblings.

At least in our second case the syndrome seems to include a hemolytic process. Definite decreased erythrocyte survival time when the patient's erythrocytes were injected into the circulation of a healthy person suggest an intracorpuscular defect. Zetterström & Strindberg (21) reported in 1958 two cases of "Sporadic Congenital Spherocytosis Associated with Congenital Hypoplastic Thrombocytopenia and Malformations". In our case 2 no spherocytosis or enzymatic defect were demonstrated. Increased susceptibility to infections in case 2 may be due to low serum levels of IgG, corticosteroid therapy and/or splenectomy. Although the thymus in case 2 was very small and atrophic this may presumably be secondary to a protracted wasting disease.

Fanconi anemia is considered to be due to an autosomal recessive gene with variable penetrance (11 19 20). A genetic basis for the occurrence of multiple cases of thrombocytopenia also seems unquestionable. Actually

Acta Pædiat Scand 59 192–198 1970

SODIUM POTASSIUM AND WATER CONTENT OF HUMAN FETAL AND MATERNAL PLASMA AND RED BLOOD CELLS

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The human fetal erythrocyte differs from its adult counterpart not only in that it contains predominantly haemoglobin F but also in other respects its mean life span is shorter presumably owing to factors inherent in the red blood cell (5) it is larger than the adult one (25) and its osmotic resistance, which is low during early fetal life increases towards partus (24) Important quantitative differences in lipid composition between the fetal and the adult erythrocyte have also been reported (6 13) Since almost all human erythrocyte lipids reside in the haemoglobin free ghosts (and therefore presumably in the membrane) functional differences between fetal and adult cells e g in electrolyte transport may be expected In this connexion intracellular concentrations of sodium and potassium are of obvious interest

McCance and Widdowson analysed erythrocytes from three human fetuses 18 to 20 weeks old and found a slightly lower potassium concentration in fetal red blood cells than in adult red blood cells (20) It was suggested that fetal cells were less able than adult cells to maintain a high potassium concentration gradient across the red cell membrane (30 31) However the potassium concentration in erythrocytes from newborns has been reported to be equal to or even greater than, that in adult red blood cells (1 23 32) Thus available data seem to suggest ontogenetic alterations in the intracellular cation concentrations in human fetal erythrocytes during the latter half of gestation but further information was considered necessary on this point

It has been reported that the potassium concentration in plasma from human fetuses at mid gestation and at term substantially exceeds the upper limit of the normal adult range (1 15 16 29 30) In a recent review it is stated the fetus seems to flourish with a concentration of potassium in its extracellular fluids far above that which is usually regarded as being the most efficient for the adult (31) However several reports have described a plasma potassium level in cord blood from newborns within or only slightly above the adult range (8 21 22 23) Furthermore in a recent study the plasma potassium concentration in umbilical artery of mid term human fetuses was found to be within the normal adult range and only slightly above the maternal level (27) It was therefore considered of great interest to reinvestigate the question of plasma potassium level in human fetuses and newborns with special reference to various factors in the sampling procedure which might explain discrepancies between earlier results The present report describes determinations of sodium and potassium in plasma and erythrocytes from human mid term fetuses and newborns and their corresponding mothers and from 1 day old infants

Table 1. Sodium and potassium in plasma and erythrocytes (RBC)

mEq/kg mean ± S.E.M.

	Plasma		RBC		
	K	Na	K	Na	
I Mid term fetus	4.81 ± 0.20	140.0 ± 0.7	99.9 ± 2.3	5.55 ± 0.41	n = 6
II Mother (fetus)	4.31 ± 0.09	135.1 ± 0.9	94.0 ± 1.6	7.26 ± 0.45	n = 6
III Newborn infant	4.59 ± 0.12	143.0 ± 1.3	97.0 ± 1.1	9.63 ± 0.58	n = 10
IV Mother (newborn infant)	3.66 ± 0.07	140.2 ± 1.1	93.5 ± 0.7	7.03 ± 0.28	n = 10
V 1 day-old infant	4.63 ± 0.04	140.3 ± 0.3	97.3 ± 0.5	10.26 ± 0.25	n = 10

RBC values corrected for 4.5 trapped plasma and 3.6 lost water during washing and centrifugation
Sodium and potassium values as expressed above (mEq/kg) may be converted to mEq/l wet weight by multiplying by 1.08 for red blood cells and by 1.07 for plasma
Highly significant differences (p 0.001)
Plasma K Newborn infant > Mother (newborn infant)
RBC Na Mid term fetus < Newborn infant
Mid term fetus < 1-day-old infant
Newborn infant > Mother (newborn infant)

MATERIAL AND METHODS

Collection of blood samples

Blood was collected from a series of 10 newborns at normal deliveries without clinical signs of intra uterine asphyxia. The birth weights ranged 3000-4750 g and the Apgar score in the first 60 sec 9-10. The blood sample was aspirated from one umbilical artery immediately after delivery of the infant concomitantly with collection of blood from a maternal cubital vein. The blood samples were brought into heparinized glass tubes and the analysis was started at once.

A second series consisted of blood samples from 10 healthy newborns taken on the day after a normal delivery (age 5 -29 hours, birth weights 30.0-4340 g). Blood was obtained by catheterizing the umbilical vein up to the inferior caval vein after e[illegible] sion of the umbilical stump. Samples were only taken from vigorous infants without clinical signs of jaundice.

A third group of samples were taken at legal abortions performed by laparotomy. P.B. narcosis was given to the mothers with thiopental sodium, nitrous oxide and fluothane. The gestational age of the fetuses ranged 16-22 weeks and the fetal crown-heel length 17-33 cm. No fetal malformation or discoloration of the amniotic fluid was seen in this group. As soon as possible after incision of the uterus loop of the umbilical cord was clamped. With the fetus left in utero one umbilical artery was catheterized and blood samples aspirated in portions of 1-2 ml. The exact time from uterine incision to collection of each blood specimen was noted. Only cases where this time to first sample did not exceed 3 min were included. In several instances only blood for a first sample could be obtained. Because of difficulty of getting enough fetal blood determinations of water content in plasma and erythrocytes in this series were performed on samples of mixed blood from both umbilical vein and artery. A blood specimen was aspirated from a cubital vein of the mother concomitantly with the incision of the uterus.

Determination of sodium and potassium

Plasma and erythrocytes were immediately separated by centrifugation at room temperature (2000 g, 3 min). Samples showing haemolysis were discarded. Plasma was diluted 51 times (100 µl + 5 ml dist water) for analysis for potassium and a further 11 times (1 ml of the diluted plasma + 10 ml dist. water) for analysis for sodium.

After the initial centrifugation plasma and buffy coat were removed and the erythrocyte washed twice in an equal volume of choline Ringer solution composition (mM): choline-Cl 146, MgCl 1, CaCl 1, NaCl 5, ortophosphoric acid 2.5, Tris buffer to pH 7.4. This solution was used because Na-efflux from erythrocytes is minimum at an external Na concentration of approximately 5 mM (12). After each resuspension in the choline Ringer the erythrocytes were collected by centrifugation at room temperature (2000 g, 3 min). The amount of plasma trapped among the erythrocytes at this centrifugal force is 4.5%, as estimated from sucrose-C de terminations (7). A [illegible] of 4.5% was used when correcting the determinations of intracellular sodium and potassium concentration for extracellular trapped fluid among the packed erythrocytes.

Extension of centrifugation time beyond 3 min did not change the amount of trapped extracellular fluid as judged from determinations of intracellular water content. Washing the erythrocytes twice was found to be sufficient, since further washing did not alter the value found for intracellular sodium concentration. The weight of 100 µl washed erythrocytes was 108.1 ± 3.3 mg, n = 20 (mean ± S.D.) with no significant difference between fetal and adult erythrocytes. The washed erythrocytes were mixed thor

oughly and haemolyzed (100 μl erythrocytes + 5 ml dist water) This haemolysate was used directly for sodium determination and diluted a further 11 times for determination of potassium

Sodium and potassium were measured in a flame photometer (Unicam) Interference in the potassium analysis by sodium was avoided by the use of a Wratten 35 filter and of a sodium concentration in the potassium standards comparable to that in the samples No interference by potassium was noted in sodium determinations Variations in phosphate concentration in the samples were found to have no influence on the potassium or sodium determinations All sodium and potassium analyses were carried out in quadruplicate The intracellular concentration of potassium in washed erythrocytes was 3 6±0 7% (mean±S E M quadruplicate samples from nine individuals) greater than in unwashed erythrocytes The measured values of intracellular cation concentrations were therefore corrected with a factor of 3 6% for water lost during washing and centrifugation

Determination of intracellular water

The water content of plasma and whole blood was determined by weighing 100 μl samples before and after drying at 100-110 C for 4 hours to constant weight The intracellular water (in mg/100 mg wet erythrocyte weight) was then calculated according to the formula

Intracellular water = [100 *A*–*B* (100–*Ht*)]/*Ht*

where *A* = water in whole blood *B* = plasma water (in mg water/100 mg whole blood and plasma respectively) and *Ht* = haematocrit value in per cent corrected for trapped plasma As the centrifugal force in the haematocrit centrifuge used was approximately 10 000 g a correction figure of 2 5 was used for trapped plasma (2) Determination of intracellular water was carried out in duplicate The maximum difference between two measurements was 1 8 of the mean

All chemicals used were of analytic grade Double distilled water was used All glass and polyethylene material was washed in 6 M hydrochloric acid and double distilled water before use The Student s *t* test was used in the statistical analysis

RESULTS

Sodium and potassium in plasma (Table 1)

In the two groups of women plasma potassium and sodium concentration was within the normal range (11 17) The potassium concentration in plasma from fetuses newborns and 1 day-old infants lay close to the upper limit for adults The difference in plasma potassium concentration between the newborns and their mothers was highly significant but not between the fetuses and the corresponding mothers

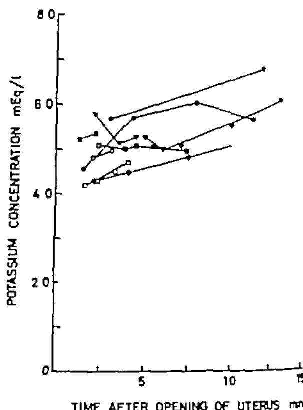


Fig 1 Potassium concentration in umbilical artery plasma Successive samples obtained from nine human mid term fetuses at different times after opening of uterus

In order to study whether the interval between uterine incision and collection of the blood influences the measured fetal plasma potassium value successive samples of blood were obtained from nine fetuses by catheterizing one umbilical artery as soon as possible after opening of the uterus (see Fig 1) The lines connecting the fetal plasma potassium values obtained in this way were extrapolated to zero time i e to the moment of uterine incision when the maternal blood sample was taken The first sample from each fetus is possibly subject to errors not likely to influence the following samples notably a local potassium release from the umbilical vessel wall at the site of catheter insertion The extrapolation was therefore started at the lowest potassium concentration measured in each series Fig 2 illustrates the difference between fetal and maternal plasma potassium concentration

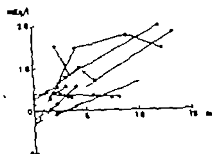


Fig. 2. Difference between fetal and maternal plasma potassium concentration as a function of time after opening of uterus. Same cases as in Fig. 1. *Ordinate:* Difference between plasma potassium concentration in fetal sample taken at the time indicated and plasma potassium concentration in maternal sample taken at the moment of uterine incision. *Abscissa:* Moment of collection of fetal sample, minutes after opening of uterus. In all except one case the umbilical cord was clamped before the first sample was obtained. In case D the cord was not clamped.

extrapolated to zero time. In none of the nine cases did this difference exceed ±0.5 mEq/l. It is reasonable to conclude that under normal conditions the potassium concentration in the fetal circulation closely reflects the potassium level in the maternal circulation.

Sodium and potassium in erythrocytes (Table 1)

Intracellular potassium did not differ significantly between any two of the five groups of



Fig. 3. Changes in intracellular sodium (----) and potassium (——) concentration in fetal erythrocytes as a function of time after opening of uterus. Values from first sample in each series taken as references. Note lowering of intracellular potassium concentration in all three cases.

Table 2. *Water in plasma and erythrocytes* (RBC)

mg H_2O/mg plasma or wet cell weight ±s.e.m.

	Plasma	RBC
I Mid term fetus	96.4±0.2 n=6	63.8±0.6 n=6
II Mother (fetus)	91.7±0.2 n=6	64.2±0.6 n=6
III Newborn infant	93.1±0.2 n=5	66.4±0.4 n=5
IV Mother (newborn infant)	91.1±0.2 n=8	65.3±0.6 n=8
V 1-day-old infant	92.7±0.2 n=7	63.4±0.3 n=6

erythrocytes. Intracellular sodium concentration was lowest in fetal erythrocytes and highest in erythrocytes from newborns and 1-day old infants. The difference between sodium concentration in fetal and newborn erythrocytes and between newborn and corresponding maternal erythrocytes was highly significant. In three mid term fetuses included in Table 1 intracellular cation content was determined in several blood samples obtained in quick succession from the umbilical artery within 1–9 min after the opening of uterus (Fig. 3). The potassium content of the erythrocytes decreased in all three cases, whereas the sodium concentration rose slightly in two of the cases during this period of blood sampling.

Water in plasma and erythrocytes (Table 2). No difference in intracellular water content was found between erythrocytes from any two of the five groups. However, the water content of plasma differed between the groups in the following order: mid term fetuses > newborns > one day-old infants > mother (fetuses) = mother (newborns). This is most probably explained by the unequal concentration of protein in the plasma groups (4).

DISCUSSION

Methodological considerations

At the analysis of washed erythrocytes, it is important to know whether the washing procedure alters the content of the cells. The results of Valberg *et al.* (28) indicate that washing of erythrocytes with magnesium chloride or choline Tris buffer does not interfere

with the determination of the intracellular potassium The use of washed erythrocytes for the determination of sodium has been criticized (19 26) However as the easily exchangeable fraction of intracellular sodium has been found to consist of plasma sodium (see discussion in (2)) the objections to the use of washed erythrocytes for determination of intracellular sodium are no longer valid Garrahan & Glynn (12) measured a sodium efflux of about 0 6 mmole/litre cell/hour from human adult erythrocytes into choline Ringer solution of the same composition as that used in the present investigation With the same choline Ringer the sodium influx was found to be 0 2 mmole/litre/hour (12) If we apply these figures to erythrocytes used in the present investigation a resulting net efflux of sodium of about 0 1 mmole per litre cell volume would occur during the washing procedure used which lasted for about 10 min

To judge from determinations of intracellular potassium concentration in the present investigation cell volume was reduced approximately 3 6 during washing of the cells It was considered of importance however to perform analysis of both intracellular sodium and potassium on identically treated cells therefore washed cells were used for both types of determination The procedures used in this investigation for obtaining erythrocytes for sodium and potassium analysis are thus adequate for the present purpose and the results after indicated corrections are comparable with intracellular cation determinations in which trapped plasma is estimated by means of small molecule tracers such as sucrose-^{14}C

Sodium and potassium plasma concentrations in fetuses and newborns

The sodium concentration in plasma from fetuses and newborns was found to lie within the normal adult range (17 11) in accordance with earlier reports (23 27 29 30) The present investigation also confirms the earlier studies which show a plasma potassium level in fetuses and newborns only slightly above the maternal level (8 14 22 23 27) Furthermore, the present results indicate that an increase of plasma potassium concentration in the umbilical artery occurs after even a brief period of disturbance of the umbilical circulation, in agreement with the conclusion reached by James (14) The rapid increase in plasma potassium cannot be ascribed to a potassium efflux from erythrocytes *in vitro* after collection of blood samples, Westin *et al* having shown that in the absence of visible haemolysis the interval between collection of fetal blood and separation of plasma from erythrocytes does not influence the plasma potassium value (29) *In vivo* however in the present investigation a rapid release of potassium from the erythrocytes to the plasma occurred after intervention with the umbilical circulation which may account at least partly for the observed increase in fetal plasma potassium concentration It is thus probable that the elevated plasma potassium concentrations previously found in human fetuses prematures and newborn infants do not reflect the normal intrauterine level but rather an effect of blood sampling and/or the influence of labour stress notably *via* a decrease in pH (9 18) causing a shift of potassium from intra to extracellular fluids This is supported by the finding that plasma potassium levels decrease during the first 15 min (27) as well as the first 24 hours (1) of extra uterine life

Sodium and potassium gradients across fetal newborn and maternal red cell membranes

The potassium and sodium concentration in erythrocytes of the two female groups in the present investigation fell within the normal adult range (3 7 10 28) No significant difference in intracellular potassium concentration was found between any two of the five groups studied This is in accordance with an earlier report that the potassium concentration in erythrocytes from newborns and their mothers is equal (23)

In the present study intracellular sodium concentration was highest in newborns and 1-day old infants and lowest in mid term fe-

tuses These differences in sodium concentrations remain unexplained It is of interest that Österlund too found a higher sodium concentration in erythrocytes from newborns than in red blood cells from their mothers (23) However in his study the absolute values for intracellular sodium concentrations were much higher than in the present investigation which might be explained by the fact that no correction was made for trapped plasma among the erythrocytes

From the present results it is evident that red blood cells from mid term fetuses newborns and 1 day old infants have approximately the same capacity to develop ionic gradients across their cell membranes as adult erythrocytes This conclusion is not compatible with the results of McCance & Widdowson (20) However their findings that the sodium concentration was higher and the potassium concentration lower in fetal than in adult erythrocytes are open to criticism for the following reasons The number of measurements on human fetal erythrocytes were few figures for statistical deviations in the material were not given and the sodium concentration reported also for adult human erythrocytes was higher than generally stated Moreover the authors found a higher water content in fetal than in adult erythrocytes whereas no such difference was found in the present investigation It should be noted that if any extracellular water is included in the figure for intracellular water it will imply a considerable error in the calculation of intracellular sodium concentration each percentage unit of trapped plasma erroneously included in red blood cell volume increase the figure for intracellular sodium by about 20

SUMMARY

Determinations were made of the sodium potassium and water content of plasma and of washed erythrocytes in blood from human mid term fetuses and their mothers from newborns and their mothers and from 1-day-old infants

No significant differences in the plasma sodium concentration were found between any two of the five groups of samples Plasma potassium concentrations in fetal and infant groups were slightly higher than in plasma from their respective mothers (4 91 and 4 68 mEq/l in plasma from fetuses and newborn infants respectively) However plasma potassium in successive samples from the umbilical artery in mid term fetuses rose with time after interference with the umbilical circulation The difference between mid term fetal and maternal plasma potassium extrapolated backwards to normal intra uterine conditions i e to the moment before opening of the uterus was less than ± 0 5 mEq/l Factors that are possibly responsible for earlier reported high plasma potassium values in fetuses and newborns are discussed

The mean intracellular potassium concentration in the five groups of erythrocytes studied varied between 93 5 and 99 5 mEq/kg no significant difference was found between any two of the five groups Sodium concentration was higher in erythrocytes from newborn infants and 1 day old infants than in those from the other three groups

It is concluded that the fetal plasma potassium concentration at mid term closely reflects the maternal level and that red blood cells from human fetuses at mid term and at term are capable of generating ionic gradients across the cell membrane of the same magnitudes as the adult red blood cells

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REFERENCES

1 Acharya, P T & Payne W W Blood chemistry of normal full term infants in the first 48 hours of life *Arch Dis Childh* 40 430 1965

2 Beilin L J Knight G J Munro Faure A D & Anderson J The measurement of sodium concentration in human red blood cells *J Gen Physiol 50* 61 1966
3 — The sodium potassium and water contents of red blood cells of healthy human adults *J Clin Invest 45* 11 1966
4 Bergstrand C G & Czar B Paper electrophoretic study of human fetal serum proteins with demonstration of new protein fraction *Scand J Clin Lab Invest 9* 277 1957
5 Bratteby L E Garby L Groth T Schneider W & Wadman B Studies on Erythrokinetics in Infancy XIII The mean life span frequency function *of red blood cells formed during foetal life Acta Paediat Scand 57* 311 1968
6 Crowley J Ways P & Jones J Human fetal erythrocyte and plasma lipids *J Clin Invest 44* 989 1965
7 Czaczkes J W Ullman T D Ullman I & Bar Kochba Z Determination of the red blood cell content of water sodium and potassium in normal subjects *J Lab Clin Med 61* 873 1963
8 Earle D P Bakwin H & Hirsch B The plasma potassium level in the newborn *Proc Soc Exp Biol Med 76* 756 1951
9 Engstrom L Karlberg P Rooth G & Tunell R *The onset of respiration A study of respiration and changes in blood gases and acid base balance* Association for the Aid of Crippled Children New York 1966
10 Funder J & Wieth J O Potassium sodium and water in normal human red blood cells *Scand J Clin Lab Invest 18* 167 1966
11 Ganrot P O Laurell C B & Nosslin B *Kompendium i klinisk kemi* Studentlitteratur Lund 1968
12 Garrahan P J & Glynn I M The behaviour of the sodium pump in red cells in the absence of external potassium *J Physiol 192* 159 1967
13 Hollán S R Szelényi J G Breuer J H Medgyesi G & Soter V N Differences between the structure and function of human adult and foetal erythrocytes *Fol Haematol 90* 126 1968
14 James L S Acidosis of the newborn and its relation to birth asphyxia *Acta Paediat Scand 49* 17 1960 Suppl 122
15 Keitel H G The concentration of potassium in the plasma *Amer J Dis Child 97* 583 1959
16 Kerpel Fronius E Nagy L & Bata G Kalié mie et potassium trésulaire du nouveau né *Arch Franc Pédiat 19* 386 1962
17 Kirschbaum T H & DeHaven J C Maternal and fetal blood constituents *In* N S Assali (ed) *Biology of gestation vol II The fetus and neonate* Academic Press New York and London 1968
18 Koch G & Wendel H Adjustment of arterial blood gases and acid base balance in the normal newborn infant during the first week of li *Biol Neonat 12* 136 1968
19 Maizels M & Remington M Cation exchange of human erythrocytes *J Physiol 145* 641 19
20 McCance R A & Widdowson E M The effect of development anaemia and undernutrition on the composition of the erythrocytes *Cl Sci 15* 409 1956
21 Newman R L Serum electrolytes in pregnancy parturition and puerperium *Obst Gyn 10* 1957
22 Overman R R Etteldorf J N Pass I N Horn G B *Pediatrics 7* 565 1951
23 Österlund K A comparative investigation of concentration of certain electrolytes in maternal and cord blood *Ann Paediat Fenn 1* Suppl 42 1955
24 Sjölin S The resistance of red cells in vi *Acta Paediat Scand 43* Suppl 98 1 1954
25 Smith C H *Blood diseases of infancy and childhood* C V Mosby St Louis 1960 p
26 Streef G M Sodium and calcium content of erythrocytes *J Biol Chem 129* 661 1939
27 Thalme B Electrolyte and acid base balance in fetal and maternal blood *Acta Obstet Gyn Scand 45* Suppl 8 7 1967
28 Valberg L S Holt J M Paulson E & Szivek J Spectrochemical analysis of sodium potassium calcium magnesium copper and zinc normal erythrocytes *J Clin Invest 44* 379 19
29 Westin B Kaiser I H Lind J Nyberg R Teger Nilsson A C Some constituents of umbilical venous blood of previable human fetus *Acta Paediat Scand 48* 609 1959
30 Widdowson E M & McCance R A The effect of development on the composition of the serum and extracellular fluids *Clin Sci 15* 1956
31 Widdowson E M Growth and Composition the Fetus and Newborn *In* N S Assali (ed *Biology of Gestation vol II The fetus and neonate* Academic Press New York and Lond 1968 p 1
32 Zipursky A LaRue T & Israel L G The in vitro metabolism of erythrocytes from newborn infants *Can J Biochem Physiol 38* 727 19

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SOMATIC DEVIATIONS IN NEWBORN AND OLDER MONGOLOID CHILDREN

A Follow-up Investigation

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We know that the most characteristic features (the cardinal signs) of newborn (1) and older mongoloid children (2) are not identical, but it is not clear how and when the various signs change. The approach to this problem can best be made by investigating a random material of mongoloid children. This investigation should then be followed up for many years. Because many mongoloid children die in infancy, the material must be collected during the newborn period. No such investigation has been reported earlier. A limiting factor has been the difficulty in diagnosing mongolism in some of the mongoloid newborns, which mainly depends on the large variation that exists. This limiting factor vanished when the development of the cytogenetic analysis increased the diagnostic possibilities.

This article gives an account of a material of newborn mongoloid children who were investigated several times up to the age of 6 years.

MATERIAL

From July 1, 1961 to June 30, 1962, all newborn suspected mongoloids in southern Sweden (Skåne, Småland, Halland and Blekinge) were examined by the author (1). During this period 25 083 children were born, of which 43 were suspected as being mongoloids. Renewed clinical examinations and chromosome analyses could exclude the diagnosis of mongolism in five instances. Thus, 38 had an extra G chromosome. The *found frequency* 0.15% (1/660) agrees with earlier investigations carried out in Europe (3). By various control methods I could be certain that all mongoloids born during the relevant period had been reported. Of the five who were not mongoloids, one had an eyelid defect (microblepharism), one showed signs of thalidomide injury with among other things seriously dysplastic ears, and one had a flat facial profile and an abundance of neck skin. The other two infants were severely dysplastic and later showed signs of mental retardation.

MORTALITY AND CARDIAC MALFORMATIONS

During the first 6–7 years the material was reduced by 16 children, 15 *of* whom died before the age of 6 months. Autopsies were performed on 10 of them, all except one being performed at a pathological institution. All 10 were found to have had congenital vitia: one had an atrial septal defect, two had ostium atrioventricularis communis, and the remaining seven had an uncomplicated ventricular septal defect. In the newborn period a cardiac X-ray was taken in nine of the children who were autopsied. A deviating heart configuration was found in seven of them, while in two the findings were uncertain. As a comparison it can be mentioned that 18 mongoloid children without cardiac symptoms in the newborn period or later were also examined by X-ray in the newborn period. The heart configuration was considered normal in 13 cases, uncertain in two, and abnormal in three.

Another two of the deceased children had shown signs of cardiac malformation, *inter alia* harsh systolic murmur and abnormal heart configuration during the X-ray examination. *Of the 15* deceased infants 12 thus had a congenital heart defect.

These 12 children had displayed the following signs of cardiac malformation. Four of them had a harsh systolic murmur as newborn, whereas in two others the murmur did not appear until the age of 14 days

In one child who also had cyanosis only a weak systolic murmur could be detected This however disappeared after two months *Three other children* manifested cyanosis and dyspnoea following exertion, but otherwise had a normal physical finding whereas another infant had only an accentuated second sound One child died 10 hours after birth

A new X ray examination of the heart supplemented by an EKG was carried out at the age of 12 months in four subjects because the examination *in the newborn period could not exclude cardiac malformation* Here obvious signs of a congenital vitium were found in two children and it was not until they were 12 months old that the systolic murmur was heard Both children are still living at 6 years of age One of them has suffered several severe episodes of illness with high fever and signs of heart incompensation Thus 14 (12+2) of the 38 children (37) *in this mongoloid material have been found to have* a cardiac malformation Only three of those who died before reaching the age of one year had no clinical signs of congenital heart malformation The cardiac X rays were normal and no heart murmur could be detected However it was noted that these children too became breathless in connection with feeding

An infection usually bronchopneumonia or gastro*enteritis was a contributory cause of death in most of* the children In some cases only fever was reported The others who died without signs of infection had severe cardiac malformations (four cases) and duodenal atresia plus cardiac malformation (one case) Death also often occurred quickly two died in their homes and three on the way to the hospital or just after arrival

After the first year only one of the children had died The cause of death was leukemia which is a known complication of mongolism Thus 22 of the original 38 mongoloid children are still alive after 6 years

METHOD

The 22 children had first been examined as newborn and then again at the ages of about 1 3 and 6 years *by the author (One child was examined by another* person at the 3 and 6 year controls) Various external mongoloid signs were appraised Head circumference cephalic index length weight and chest circumference were also recorded Repeated X rays of the skeleton could also be performed in some of the children The children were also appraised according to Vineland's Social Maturity Scale Only certain parts *of this follow* up investigation will be commented on in this article

THE NURSING SITUATION OF THE MONGOLOIDS

Since early infancy three of the 22 children have been cared for *outside of their own homes* One child has been in a nursing home since the age o years but visits its own home during holid Another child was placed in a nursing home at t age of 6 years Of the 22 children 17 are still a years of age in their own homes

RESULTS

Not less than 20 of the mothers of the mongoloid children were 35 years old or mo at the time of the birth of their child t average age is high 34 7 years Seven of t children were premature The average bi weight was 3060 g, i e c 4 hg less than t average normal newborn This can only part be explained by the pregnancy period usual being one week shorter than normal

Various signs in the newborn period

The general phenotype of the newborn mong oid children was described in an earlier wo (1) Hence only a short description of t various signs of the newborn mongoloid will given here

The facial expression of the newborn often quite characteristic small mouth a nose oblique palpebral fissures and the e pressionless mimic The facial appearance the mongoloids however is far from ide tical One of the signs first noticed is t *pronounced pallor Their passivity* is also stri ing They lack the spontaneous motility of t normal child The Moro reflex is usually a sent Muscle hypotonia is common and mai noticed by the one who nurses the child T skin is pasty the neck skin being particular abundant Hyperflexibility is another obvio sign The children cry *seldom and then* weak The unusual crying often creates suspicion *the mother that all is not well*

The most usual eye signs are iris spots a *the oblique palpebral* fissures The newbor have no true epicanthus but their medial e angle is blunt and not normally pointed Co siderable information can be obtained by e amining the profile, which is flat The nas bridge especially is hardly developed The hea is round and lacks the powerful occiput th

characterizes normal newborn its circumference is small (average value = 33.0 cm). The ear is small and lacks distinct contours. It does not have the normal oval shape but is round or almost square. Usually only the upper part of the helix is folded. The mouth, similar to the nose, is small and the corners usually droop. The tongue often protrudes although this is not so obvious. In scarcely half of the children the palm of the hand shows a four finger line but in almost all the palms are poorly differentiated with many small furrows and indistinct main furrows. The short broad mongoloid hand is difficult to appraise in the newborns because they practically always have their hands clenched. The nails may be hyperconvex. There is often a considerable space between the first and second toes. The X-ray examinations showed that more than half of the children had a dysplastic middle phalanx of the little finger (see Fig. 1) and most had a broad low pelvis. This deviating pelvic form can also be expressed by the use of an index, Caffey's index, which is half the sum of the acetabular and ilium angles. The average value for the mongoloid children was 69°, which was 10° less than for normal controls.



Fig. 1. Schematic drawings of the skeleton of the fifth finger from the same mongoloid patient examined as a newborn and on three other occasions showing a normalization of the dysplastic middle phalanx (arrow).

Signs that had diminished or disappeared[1] *(cf. also Fig. 2)*

At the follow-up investigation the following signs showed a reduced strength of frequency. The obvious pallor of the newborns was still present at the age of 6 years but was too weak to be of diagnostic value. The newborns had a weak, characteristic cry but all screamed normally at 6 years of age. Another characteristic sign in the newborn, the abundant pasty neck skin, also tends to disappear: it was observed in only three children at the 6-year investigation. The skin also seemed less pasty at this age. There were less children whose tongues protruded. This sign is more noticeable when the children begin to suck their tongues after the age of 12 months but later disappears almost completely. Only two of the 6-year olds had a protruding tongue. The oblique palpebral fissures are very characteristic of the mongoloids: they become more obvious when

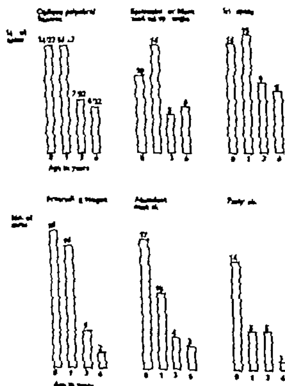


Fig. 2. The frequencies are given for some mongoloid signs in a mongoloid material (23 cases) which have been examined since the newborn period. Before the last two investigations the material was reduced by one patient.

[1] The frequencies are given only for the children who survived infancy.

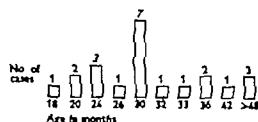


Fig 3 Age of walking

the child cries Among the newborn, 14 out of 23 had this sign but the figure was reduced to six at the age of 6 years Another eye sign the iris spots, also showed the same tendency *to disappear* and was found in fewer 6 year olds

The epicanthal fold is not developed in the newborn When the children were 12 months old however a distinct epicanthus was observed in 14 out of 23 children but later this again reduced in frequency At 6 years of age six children had this less valuable sign which is also common in normal children Muscle hypotonia is another feature that disappears Obvious hypotonia was noted in 13 of the newborn and moderate hypotonia in only five of the 6 year olds The hyperconvex nails of the newborn had become normal at the 6 year control In some children with a dysplastic *middle phalanx of the little finger* a tendency for normalization was noted at the follow up investigations even though one or another of the middle phalanges were not wholly normal The most obvious example is shown in Fig 1 An X ray examination of the hand skeleton could be made in only 15 of the 6 year olds

Snuffly breathing is another feature that characterizes the mongoloid children During the first year of infancy children with snuffly breathing increased from 5 to 10 Thereafter the breathing improved and at 6 years of age all breathed normally The most natural explanation is that the children s abnormal susceptibility to infection decreased considerably from the age of 4 years on This applies also to the tendency for blepharitis In 6 years of age suffer a slight attack of blepharitis with a severe cold

Signs that had not changed

The frequency of hyperflexible children h not changed up to the age of 6 years althou the flexibility in the joints was more pr nounced in the newborn Equally many of t older children seem also to have a flat fac *profile The external ear had not changed* shape to an extent worth mentioning Howev the ear was no longer flabby as in the newbor instead its contours were more firm Most the mongoloids, both as newborn and as year olds had a short, broad hand The fou finger line occurred with the same frequenc the different ages

Signs that had developed or had become strengthened

The 6 year-old children often have dry sk and their cheeks have a tendency toward re ness especially after remaining out-of door The round head shape of the newborn changed and the 6 year old children are i stead characterized by a flat occiput (16 out 22 children) Short stature is more obvious the older children The average value for th 6 year old mongoloids was 108 cm Th mongoloids grow more slowly than norm *children It can also be noted in the mongo* oids that the thorax often has a deviant form it is usually broad and has a slight de pression at the lower part of the sternum I the older mongoloid children it is more notic ible that the foot is short clumsy and so what splayed The space between the first an second toes is not only large but the big to shows a tendency to point medially In th newborn only the large space between the fir and second toes was noted The difference i *partly a result of the* load that the foot is sub jected to The bent little fingers are also mor conspicuous in the older children In some o the children the tongue begins to develop fis sures The pelvis becomes increasingly dysplas tic The mean value for Caffey s index has thus decreased by 21 since the newborn period

Some observations on the functions of the 6 year old children

Only one child was so retarded that even at 6 years of age it could not understand what was said to it. One girl impressed us by being able to speak almost normally. Most of the children spoke in a manner that could be understood only by their relatives, but six of them could compose quite understandable sentences. Almost as many (five) could not speak, but only made special sounds. Most of them were somewhat hoarse.

No less than 13 out of the 22 children could feed themselves. Somewhat more—16—could get water to drink, and 13 could ask for permission to go to the toilet. Three more could be managed by careful attention and eight of them had nocturnal enuresis. Fourteen children were unable to descend a staircase normally. The large variation in development in this material can be demonstrated by the debut of walking, which is shown in Fig. 3. Two of the children who did not walk until after the age of 4 years deviated also in other ways from the other children. One was also poorly developed in other respects, i.e. lacked the faculty of speech and displayed much stereotypy, whereas the other child had congenital cataracts and concomitant blindness.

DISCUSSION

The material consists of all the mongoloid children born in a large geographical area, therefore, despite its smallness, it should be representative of mongoloids in general. Comparisons were made with larger materials, especially concerning data that could be extracted from case records and autopsy reports, and have also shown good agreement. Forssman & Akesson (4) found in a larger Swedish material that the mothers' average age at the birth of the mongoloid child was 35.4 years; this does not differ appreciably from the average in the present material, 34.7 years. The debut of walking was compared with the results in the material of Levinson *et al.* (5). There is a slight discrepancy between the materials. More mongoloids in their material began to walk at 2 years of age, while in the present material the debut of walking usually occurred at the age of 2 1/2 years. There is, however, a wide distribution pattern in both materials. Rowe & Uchida (6) investigated the most representative mongoloid material; this, however, is not altogether free from objections. Their intention was to determine the cardiac malformation frequency. They found that 40% of the mongoloids had cardiac malformations; this corresponds to 37% in the present material. Earlier it was maintained that A.V. communis is the dominant defect in mongolism. Berg *et al.* (7), however, in a large autopsy material could show that the ventricular septal defect is the most common finding in mongoloids. A.V. communis is more common in mongoloids than in normals. Also in the present material the ventricular septal defects dominated and A.V. communis occurred in no less than 2 of the 10 autopsied. Esen (8) showed that 9 out of 12 mongoloid children with cardiac malformations died before 2 years of age, whereas only 3 out of 19 children without congenital heart disease died during the same period. As early as 1950 Evans (9) pointed out that mongoloids with cardiac malformations usually die between the ages of two and five months. In the present material most of the children with a congenital cardiac vitium also died before the age of 5 months (10 out of 14), whereas only 3 out of the other 24 children died during the first two years of life. The comparisons thus argue that the present material is representative of mongoloids in general.

The prognosis for the mongoloids with congenital heart malformations is thus very poor. Most die during the first year of infancy. This cardiac malformation occurs also at such a high frequency that a cardiological investigation (with *inter alia* X-rays) of the newborn mongoloid children is to the greatest extent justified.

It is usually maintained that the clinical

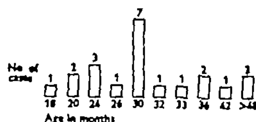


Fig 3 Age of walking

the child cries Among the newborn 14 out of 23 had this sign but the figure was reduced to six at the age of 6 years Another eye sign the iris spots also showed the same tendency to disappear and was found in fewer 6 year olds

The epicanthal fold is not developed in the newborn When the children were 12 months old however, a distinct epicanthus was observed in 14 out of 23 children but later this again reduced in frequency At 6 years of age six children had this less valuable sign which is also common in normal children Muscle hypotonia is another feature that disappears Obvious hypotonia was noted in 13 of the newborn and moderate hypotonia in only five of the 6 year olds The hyperconvex nails of the newborn had become normal at the 6 year control In some children with a dysplastic middle phalanx of the little finger a tendency for normalization was noted at the follow up investigations even though one or another of the middle phalanges were not wholly normal *The most obvious example is shown in Fig 1* An X ray examination of the hand skeleton could be made in only 15 of the 6 year olds

Snuffly breathing is another feature that characterizes the mongoloid children During the first year of infancy children with snuffly *breathing increased from 5 to 10 Thereafter* the breathing improved and at 6 years of age all breathed normally The most natural explanation *is that the children's ab*normal susceptibility to infection decreased considerably from the age of 4 years on This applies also to the tendency *for blepharitis In* 6 years of age suffer a slight attack of blepharitis with a severe cold

Signs that had not changed

The frequency of hyperflexible children had not changed up to the age of 6 years although the flexibility in the joints was more pronounced in the newborn Equally many of the older children seem also to have a flat facial profile The external ear had not changed in shape to an extent worth mentioning However the ear was no longer flabby as in the newborn instead its contours were more firm *Most of* the mongoloids, both as newborn and as 6 year olds, had a short broad hand The four finger line occurred with the same frequency at the different ages

Signs that had developed or had become strengthened

The 6 year-old children often have dry skin and their cheeks have a tendency toward redness especially after remaining out-of-doors The round head shape of the newborn is changed and the 6 year old children are instead characterized by a flat occiput (16 out of 22 children) Short stature is more obvious in the older children The average value for the 6 year old mongoloids was 108 cm The mongoloids grow more slowly than normal children It can also be noted in the mongoloids that the thorax often has a deviating form it is usually broad and has a slight depression at the lower part of the sternum In the older mongoloid children it is more noticeable that the foot is short clumsy and somewhat splayed The space between the first and *second toes is not only large* but the big toe shows a tendency to point medially In the newborn only the large space between the first *and second* toes was noted The difference is partly a result of the load that the foot is subjected to The bent little fingers are also more *conspicuous in the older children* In some of the children the tongue begins to develop fissures The pelvis becomes increasingly dysplas*tic The mean value for Caffey's* index had thus decreased by 21 since the newborn period

Some observations on the functions of the 6-year-old children

Only one child was so retarded that even at 6 years of age it could not understand what was said to it. One girl impressed us by being able to speak almost normally. Most of the children spoke in a manner that could be understood only by their relatives, but six of them could compose quite understandable sentences. Almost as many (five) could not speak, but only made special sounds. Most of them were somewhat hoarse.

No less than 13 out of the 22 children could feed themselves. Somewhat more—16—could get water to drink, and 13 could ask for permission to go to the toilet. Three more could be managed by careful attention and eight of them had nocturnal enuresis. Fourteen children were unable to descend a staircase normally. The large variation in development in this material can be demonstrated by the debut of walking, which is shown in Fig. 3. Two of the children who did not walk until after the age of 4 years deviated also in other ways from the other children. One was also poorly developed in other respects, i.e. lacked the faculty of speech and displayed much stereotypy, whereas the other child had congenital cataracts and concomitant blindness.

DISCUSSION

The material consists of all the mongoloid children born in a large geographical area, therefore despite its smallness it should be representative of mongoloids in general. Comparisons were made with larger materials, especially concerning data that could be extracted from case records and autopsy reports and have also shown good agreement. Forssman & Akesson (4) found in a larger Swedish material that the mothers' average age at the birth of the mongoloid child was 35.4 years; this does not differ appreciably from the average in the present material: 34.7 year. The debut of walking was compared with the results in the material of Levinson et al. (5). There is a slight discrepancy between the materials. More mongoloids in their material began to walk at 2 years of age, while in the present material the debut of walking usually occurred at the age of 2½ years. There is, however, a wide distribution pattern in both materials. Rowe & Uchida (6) investigated the most representative mongoloid material; this, however, is not altogether free from objections. Their intention was to determine the cardiac malformation frequency. They found that 40 % of the mongoloids had cardiac malformations; this corresponds to 37 % in the present material. Earlier it was maintained that A-V communis is the dominant defect in mongolism. Berg et al. (7), however, in a large autopsy material could show that the ventricular septal defect is the most common finding in mongoloids. A-V communis is more common in mongoloids than in normals. Also in the present material, the ventricular septal defects dominated and A-V communis occurred in no less than 2 of the 10 autopsied. Esen (8) showed that 9 out of 12 mongoloid children with cardiac malformations died before 2 years of age, whereas only 3 out of 19 children without congenital heart disease died during the same period. As early as 1950, Evans (9) pointed out that mongoloids with cardiac malformations usually die between the ages of two and five months. In the present material, most of the children with a congenital cardiac vitium also died before the age of 5 months (10 out of 14), whereas only 3 out of the other 24 children died during the first two years of life. The comparisons thus argue that the present material is representative of mongoloids in general.

The prognosis for the mongoloids with congenital heart malformations is thus very poor. Most die during the first year of infancy. This cardiac malformation occurs also at such a high frequency that a cardiological investigation (with inter alia X-rays) of the newborn mongoloid children is to the greatest extent justified.

It is usually maintained that the clinical

diagnosis of mongolism is most difficult to make during the newborn period This can be interpreted in such a way that the mongoloid phenotype is more pronounced as the child grows older The alternative explanation is that the lack of distinct signs of mental retardation in the newborn contributes to the diagnosis being so difficult to make According to the present investigation the latter alternative is the most probable one Many of the signs in the newborn are either diminished or have disappeared whereas only a few signs have been strengthened or added Thus the mongoloid phenotype is most pronounced in the new born child Many of the signs involved could only be appraised subjectively One of the few mongoloid signs that can be measured is the head circumference An earlier investigation (10) showed that the mongoloids with the smallest head circumference as newborn grew most rapidly with respect to head circumference which is also a form of normalization On the whole however mongoloids grow much more slowly than normal children At 6 years of age they are of conspicuously short stature and the picture is dominated by mental retardation especially their poor speech

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REFERENCES

1 Hall B Mongolism in newborns A clinical and cytogenetic study *Acta Paediat Scand* Suppl *154* 1964
2 Øster J *Mongolism* Danish Science Press Ltd Copenhagen 1953
3 Penrose L S & Smith G F *Downs Anomaly* J & A Churchill Ltd London 1966
4 Forssman H & Akesson H O Personal communication
5 Levinson A Friedman A & Stamps F Variability of mongolism *Pediatrics 16* 43 1955
6 Rowe R D & Uchida I A Cardiac malformations in mongolism *Amer J Med 31* 726 1961
7 Berg J M Crome L & France N E Congenital cardiac malformations in mongolism *Brit Heart J 22* 331 1960
8 Esen F M Congenital heart malformations in mongolism with special reference to ostium atrioventriculare commune *Arch Pediat 74* 243 1957
9 Evans P R Cardiac anomalies in mongolism *Brit Heart J 12* 258 1950
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ACID PHOSPHATASE ACTIVITY IN THE SYNOVIAL FLUID OF CHILDREN WITH JUVENILE RHEUMATOID ARTHRITIS

G BECKMAN L BECKMAN L GOTHEFORS and R LEMPERG

From the Departments of Clinical Bacteriology Orthopedic Surgery and of Paediatrics University of Umeå Umeå Sweden

The diagnosis of juvenile rheumatoid arthritis (JRA) is at present not easy to establish at an early stage of the disease The serological reactions are usually negative especially in monarticular cases (2 4) The diagnosis is therefore usually arrived at by the exclusion of other diseases such as tuberculosis bacterial arthritis and ulcerative colitis or by the subsequent involvement of additional joints

In a previous study of synovial fluid (1) the authors found a significantly increased acid phosphatase activity in adult patients with rheumatoid arthritis in comparison with patients with non inflammatory joint disorders Furthermore a number of patients with monarticular synovitis and high acid phosphatase activity but negative serological reactions were found to develop definite rheumatoid arthritis within 1 year

It was therefore considered of interest to examine children with different joint effusions with the aim of assessing whether determinations of acid phosphatase activity in the synovial fluids may help to ascertain early diagnosis of a rheumatoid joint disorder

MATERIAL AND METHODS

From [illegible] children with effusions in one or more joints synovial fluid was obtained by joint puncture Only cases which have been followed up for at least half [illegible] year after joint fluid examination are included in this study The diagnoses at the time of joint fluid aspiration were in these patients as follows (See also Table 1)

In 5 cases with engagements of more than one joint the diagnosis of JRA was made according to the criteria recommended by Edström (3) after exclusion of other diseases These patients had one knee and in addition one or two major joints engaged.

Seven patients had only one joint affected In two of them the diagnosis of JRA was made according to the criteria recommended by Cassidy *et al* (2) Both had a chronic synovitis of one knee joint with a duration of more than 4 months Of the remaining 5 patients 4 had a monarticular *joint effusion* of short duration and unknown etiology in either the knee or the hipjoint One patient had persisting effusion after a bacterial arthritis confirmed by bacterial culture *from the synovial fluid 1 month* earlier

In all patients standard tests for rheumatoid factors roentgenograms of the affected joints chest film L E cell test, complete blood count and paper electrophoresis of the serum proteins were made

All samples of synovial fluid were subjected to routine bacteriological examination In 7 cases total and differential white cell counts of the synovial fluid were performed Synovial biopsies were obtained in 6 cases in connection with synovectomy performed at different intervals after joint fluid examination Determinations of acid phosphatase activity were made with a naphthyl phosphate as substrate (1)

RESULTS

None of the patients had a positive serological reaction for rheumatoid arthritis The results of the chest film L E cell tests blood cell counts and serum protein electrophoresis did not indicate the presence of diseases other than rheumatoid arthritis The roentgenological findings were normal or compatible with JRA with the exception of one patient (no 9) All patients operated on with synovectomy showed at inspection of the joint a picture of extensive inflammation of the tunica synovialis The

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Table 1 *Clinical data and acid phosphatase activity*

Activity expressed in micromoles of α naphthyl phosphate hydrolyzed per ml fluid per hour The clinical diagnosis and duration of illness refers to the time of joint fluid analysis

Case no	Age (years)	Clinical diagnosis	Duration of illness	No of joints involved	Acid phosphatase	Remarks
1	8	JRA	7 years	2	1 13	
2	8	JRA	7 years	3	4 12	
Same patient 1 month after synovectomy					1 39	
3	8	JRA	3 years	2	2 52	
4	10	JRA	8 years	2	3 09	
Same patient 2 months after synovectomy					2 12	
5	13	JRA	2 years	3	1 06	
6	13	JRA	1 year	1	3 97	No further joint engaged after 2 years
Same patient 9 months after synovectomy					0 12	
7	14	JRA	9 months	1	3 97	One year later still joint effusion no further joint engaged
8	1	Monarticular synovitis	1 month	1	1 69	Op one year later Diagn JRA confirmed by synovial biopsy
9	4	Monarticular synovitis (bacterial osteo-arthritis?)	6 weeks	1	3 93	After 9 months still signs of synovitis No other joint engaged Diagnosis still uncertain
10	12	Monarticular synovitis	5 weeks	1	1 47	Op 5 months later No further joint engaged after 9 months
11	4	Transient arthritis of the hip	1 day	1	0 30	Recovered no further joint engaged after 1 year
12	7	Bacterial arthritis	1 month	1	0 32	Recovered no further joint engaged after 2 years

microscopic diagnosis was in 5 out of 6 cases examined nonspecific synovitis In case 8 a picture compatible with rheumatoid synovitis was found

Culture of the synovial fluids revealed no bacterial or viral growth at time of enzyme examination The white cell counts of the synovial fluids were variable and apparently without any correlation with the acid phosphatase activity

The acid phosphatase activity was high in the poly and monarticular cases with the initial diagnosis JRA (cases 1–7 Table 1)

The acid phosphatase activity was also high in 3 cases with monarticular joint effusions of initially unknown etiology (nos 8–10) Two of these cases (nos 8 and 10) showed persisting signs of synovitis 1 year and 5 months respectively after joint fluid examination Synovectomy was performed in both cases Case 8 showed a histopathological picture compatible with rheumatoid synovitis Patient 10 showed a picture of chronic non specific synovitis but was classified as a case of probable JRA due to the persistence of synovitis over a period longer than 4 months The third case (no 9) showed on X ray pictures 2 months after the joint fluid examination a bone destruction in the femoral epiphysis The suspected diagnosis of bacterial osteoarthritis could not be confirmed by bacterial culture After 9 months signs of synovitis still persisted, but the bone destruction showed definite regression

Two patients showed low initial values of acid phosphatase activity (nos 11 and 12) Case 11 had a coxitis which by its clinical development and rapid regress of symptoms was considered to be a transient arthritis Case 12 had a bacterial arthritis of 1 month s duration Both patients recovered completely and showed after 2 and 1 year s (respectively) follow up no further joint engagement

From 3 patients with the diagnosis JRA synovial fluid was examined after synovectomy (nos 2 4 and 6) In these cases the acid phosphatase activity decreased after operation In 2 cases (with more than one joint engaged) the decrease of enzyme activity was moderate (no 2 and 4) One patient with monarticular engagement (no 6) showed low values comparable with those found in non inflammatory joint effusions No further joint engagement was observed in this patient

DISCUSSION

The mean acid phosphatase level in 7 children with the initial diagnosis JRA (Table 2) has been compared with those found in an earlier investigation of adult patients with rheumatoid arthritis and with non inflammatory joint effusions (1) In JRA the acid phosphatase activity was high with a level very similar to that found in classic and definite rheumatoid arthritis of adults The level is significantly ($p<0.001$) higher than that found in non inflammatory joint effusions of adults

Two non rheumatoid patients (nos 11 and 12) had low enzyme levels comparable with those of adults with non inflammatory joint effusions

A high acid phosphatase activity was found also in 3 patients with an initial clinical picture of monarticular synovitis In one of these cases the diagnosis JRA was confirmed 1 year later by synovial biopsy In another case the course of the disease points towards rheumatoid arthritis In the third case the possibility of bacterial osteo-arthritis could not be excluded with sufficient certainty but the course of the disease was indicative of JRA

Elevated levels of acid phosphatase activity in the synovial fluid were found in JRA High levels were also found in monarticular chronic synovitis which later proved to be JRA Determinations of acid phosphatase activity in the synovial fluid may therefore contribute to the ascertainment of the diagnosis of JRA Thus it seems worthwhile to examine the acid phosphatase level of the synovial fluid in a larger number of children in order to assess its value in the early diagnosis of JRA

Table 2. *Acid phosphatases activity in the synovial fluid of adult patients with classic and definite rheumatoid arthritis juvenile rheumatoid arthritis and adults with non inflammatory joint effusions*

Activity expressed in micromoles of α naphthyl phosphate hydrolyzed per ml fluid per hour

Patient group	Acid phosphatase activity $M \pm s.e.$	S.D.	No. examined
Classic and definite rheumatoid arthritis adults[a]	2.86±0.20	1.68	101
Juvenile rheumatoid arthritis	2.84±0.50	1.32	7
Non inflammatory joint effusions adults[a]	0.22±0.05	0.09	17

[a] Data from Beckman *et al.* (1)

SUMMARY

The acid phosphatase activity in the synovial fluid of nine children with juvenile rheumatoid arthritis was high and of the same order of magnitude as that found in adult patients with classic and definite rheumatoid arthritis The present results suggest that determinations of acid phosphatase activity may be a useful contributory test in the diagnosis of juvenile rheumatoid arthritis

REFERENCES

1 Beckman G, Beckman, L. & Lemperg R. Acid phosphatase activity in the synovial fluid of patients with different joint disorders To be published
2 Cassidy J T Brody G L & Martel, U Monarticular juvenile rheumatoid arthritis J Pediat 70 867 1967
3 Edström G Rheumatoid arthritis and Still's disease in children Arthritis Rheum 1 497 1958
4 Laaksonen A. A prognostic study of juvenile rheumatoid arthritis Acta Paediat Scand 55 Suppl 166 1966

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Fig. 1. Bronchial artery supplied area of the lung with prominent atelectases and hyaline membranes. Age 4 days, birth weight 450 g (A 161 V2). (*a*) Microangiogram × 30. The bronchial arteries in the upper part of the *field and* ramifications to the pulmonary parenchyma. (Corresponding *arrows* in *A* and *B*.) (*b*) Serial histological section from framed area in *A*. Elastin van Gieson × 76.

The present work on the bronchial artery supply of the pulmonary parenchyma complements previous post mortem microangiographical studies on the pulmonary vasculature in IRDS. With a concentrated barium suspension Lauweryns (4) obtained poor filling of the peripheral pulmonary arteries and arterioles and interpreted the results as indicating pulmonary hypoperfusion. On the other hand Lindgren (6) and Ivemark & Wallgren (3) who used the same technique in principle as here demonstrated a normal pulmonary artery pattern in IRDS lungs. Nor are there signs of an abnormal pulmonary vein pattern in IRDS lungs (3, 5). The bulk of the evidence then is compatible with a normal vascular pattern in IRDS lungs.

Boné's (?) hypothesis in his report of the Lausanne baby that systemic artery supply of the pulmonary parenchyma prevents the development of PHM has been questioned on other grounds by Adams & El Salawy (1). They elicited evidence that the lung lobe in question was totally sequestered, i.e. had neither respiratory nor arterial connections with the rest of the lung.

Since PHM equally affects areas of the human neonatal lung supplied by systemic and pulmonary arteries there are no grounds for supposing that abnormalities in the pulmonary artery system alone constitute a primary factor in the still obscure pathogenesis of IRDS.

SUMMARY

A combined microangiographical and histological study of the bronchial arterial system

Acta Pædiat Scand 59 208–210 1970

SHORT COMMUNICATION

HYALINE MEMBRANES IN BRONCHIAL ARTERY SUPPLIED AREAS OF THE HUMAN NEONATAL LUNG

A Microangiographical and Histological Study

C A GRANT and B ROBERTSON

From the Department of Paediatric Pathology Karolinska sjukhuset Stockholm Sweden

Asymmetrical distribution of atelectasis and pulmonary hyaline membranes (PHM) in the idiopathic respiratory distress syndrome (IRDS) has been noticed on a few occasions (2 7 10) In some of these cases the asymmetry of the lesions was attributed to impairment of pulmonary lymphatic flow (7) or abnormal systemic artery supply of the unaffected lung lobe (2) However in the newborn infant small areas of the pulmonary parenchyma are normally supplied by bronchial artery branches so called bronchopulmonary arteries (9 11) In view of the possible influence of vascular aberrations on the development of PHM we have carried out the present study on the occurrence of PHM in areas of the neonatal lung supplied by bronchopulmonary arteries

MATERIAL AND METHODS

The bronchial arterial system in 10 newborn infants with IRDS and PHM was injected via the thoracic aorta with 7 5 per cent aqueous suspension of fine barium sulphate (Micropaque[1]) Bronchial artery supplied areas of the pulmonary parenchyma were identified in microangiograms representing 1500 μ thick frontal slices of the lung (for details of the microangiographic technique see 8) Serial histological sections from such areas to confirm the existence of bronchopulmonary arteries were then examined for the presence of PHM and compared with sections from other parts of the lung The birth weight of the subjects ranged from 1100 g to 2780 g and their age from 8 hours to 5 days

RESULTS

In 8 of the infants small areas of pulmonary parenchyma supplied by branches of the bronchial artery could be identified The pattern fitted in with what is normally encountered in the lung of the newborn infant i e scattered small wedges of pulmonary parenchyma usually adjacent to bronchial bifurcations are marginally supplied with filling of the peripheral alveolar capillaries In two infants the bronchial artery branches extended deeper into the wedges to supply the terminal respiratory passages as well In places there was transmission of contrast to peripheral pulmonary-artery branches through a common capillary bed Taken as a whole branches of the bronchial arteries supplied only a very minor portion of the pulmonary parenchyma Regardless of the origin of the arterial supply however the distribution of PHM and atelectasis was uniform throughout the parenchyma

There was no evidence for abnormal arterial bronchopulmonary anastomoses or arteriovenous shunts

COMMENT

The number mural structure and distribution pattern of bronchopulmonary arteries in the lungs of these infants with IRDS and PHM followed the normal pattern (8)

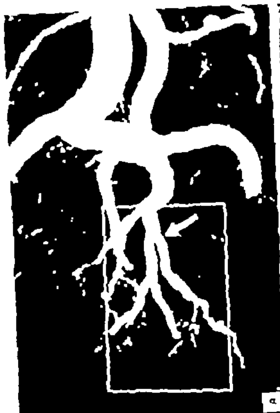




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A combined microangiographical and histological study of the bronchial arterial system

was carried out on 10 neonatal subjects dying from the idiopathic respiratory distress syndrome with pulmonary hyaline membranes The bronchial artery pattern was normal in all cases Small areas of the pulmonary parenchyma supplied by bronchial artery branches, so-called bronchopulmonary arteries were identified in 8 infants The pulmonary lesions were the same in these areas as in the rest of the lung indicating that systemic artery supply of the pulmonary parenchyma does not prevent the formation of pulmonary hyaline membranes

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8 Robertson B The intrapulmonary arterial pattern in normal infancy and in transposition of the great arteries *Acta Paediat Scand* Suppl 184 1968
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CASE REPORT

RECURRENT BACTEREMIA FOLLOWING AMPICILLIN TREATMENT OF HEMOPHILUS INFLUENZAE MENINGITIS

ROBERT A ACHTEL

From the Department of Pediatrics Yale University School of Medicine and the Yale New Haven Hospital New Haven Connecticut U.S.A

Ampicillin has been accepted as an effective therapeutic agent in the treatment of *Hemophilus influenzae* infections (1 7 11). *In vitro* sensitivity remains virtually complete (9 11). However eight cases of ampicillin failure in Hemophilus influenzae meningitis have been reported (8 10). Failure has been ascribed to inadequate cerebrospinal fluid levels of ampicillin secondary to inadequate dosage or improper route of administration in seven of these cases (2 8). In the eighth case Young *et al* (10) reported the occurrence of orbital cellulitis and bacteremia three days after a 14 day course of ampicillin suggesting inadequate antibiotic perfusion in an area where organisms had been sequestered. Subdural punctures were performed in three of the eight reported cases only one being productive of fluid which proved sterile.

The following is a case report of an infant with *Hemophilus influenzae* meningitis where ampicillin failed to eradicate the infection possibly because of the presence of left subdural effusion.

CASE REPORT

B A, a seven month-old white male presented with a generalized seizure a bulging anterior fontanelle bilateral otitis media and fever of 40 C. A lumbar puncture showed grossly purulent cerebrospinal fluid (Table 1) cultures of which subsequently grew *Hemophilus influenzae* type B. *Growth* inhibition was greater than 15 mm with a 10 μg ampicillin disc. Intravenous ampicillin therapy was begun with an initial dose of 1 g (103 mg per kg) and continued at 2 (206 mg per kg) per day administered over a ten minute period in divided doses every four hours for a period of ten days. Ampicillin was administered intramuscularly on the 11th and 12th hospital days because of the paucity of suitable veins. A second lumbar puncture was performed 48 hours after ampicillin therapy (Table 1) and cultures were sterile.

A right hemiparesis became evident by the 3rd hospital day. Transillumination was moderately increased over the left frontoparietal region. A left subdural puncture through the coronal suture yielded 1 ml of sterile fluid (Table 2). Fever declined gradually to normothermic levels by the 5th day but rose to 39 C on the 9th day. Transillumination was no longer increased and the right hemiparesis had disappeared. A purulent nasal discharge had developed but nasopharyngeal cultures revealed normal flora. A 3rd lumbar puncture performed on the 10th day (Table 1) was sterile. Tube dilutions of cerebrospinal fluid demonstrated growth inhibition of the original organisms at 1 4. The patient was afebrile by the 12th day, cultures remained sterile and ampicillin was discontinued. The patient's temperature rose to 38 C on the 13th day, a fourth lumbar puncture was performed and chloramphenicol in dosage of 100 mg kg a day was given intravenously in divided doses administered every four hours. Fever abated within 72 hours, cerebrospinal and blood cultures remained sterile and a repeat lumbar puncture was (Table 1) within normal limits. Chloramphenicol was therefore discontinued.

A low grade fever (between 38 and 38 5 C) developed within 24 hours and persisted for the next 7 days in the absence of clinical symptoms. Nasopharyngeal, blood and urine cultures were unproductive. X rays of the chest, sinuses, and mastoids and immunoelectrophoresis were within normal limits. The patient's temperature was 39 5 C on the 24th day

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A low grade fever (between 38 and 38.5 C) developed within 24 hours and persisted for the next 7 days in the absence of clinical symptoms. Nasopharyngeal, blood and urine cultures were unproductive X rays of the chest sinuses and mastoids and immunoelectrophoresis were within normal limits The patient's temperature was 39.5 C on the 24th day

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strongly suggestive of a subdural effusion Negative findings from the first subdural puncture did not rule out a subdural effusion as the needle may not have entered the fluid lake or the viscosity of the fluid may have precluded withdrawal A positive culture from the thick material obtained after left subdural puncture on the 24th day proves infection within this space These findings would support the hypothesis of sequestration of organisms in the subdural space with subsequent reseeding of blood stream The negative subdural puncture on the 37th day suggests that sterilization of this space may have occurred following combined therapy with both ampicillin and chloramphenicol or evacuation of the space

Failure of ampicillin in *Hemophilus influenzae* meningitis may be the result of inadequate perfusion of the cerebrospinal fluid by the antibiotic as a result of insufficient dosage or administration by other than the intravenous route Less frequently where adequate cerebrospinal fluid levels of an effective antibiotic are maintained relapse may be attributed to hematogenous or cerebrospinal fluid reseeding by organisms sequestered in areas which were inadequately perfused A thorough search for foci of persistent infection must include subdural puncture whenever ampicillin failure is suspected in *hemophilus meningitis*

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Table 1 *Lumbar punctures*

LP	Hosp day	TNC	Cells Gran	Lymph	RBC s	Protein	CSF sugar	Blood sugar	Culture
1	1	1660	1644	16	0	45	40	109	*H infl*
2	2	170	136	34	5	48	48	62	Neg
3	10	9	1	8	0	33	46	87	Neg
4	13	310	31	279	90	32	38	87	Neg
5	16	0	0	0	39	26	63	90	Neg
6	24	16	0	16	5	40	50	110	Neg
7	36	55	5	50	0	81	53	85	Neg

TNC – Total nucleated cells Gran – granulocytes Lymph – lymphocytes

after admission or 8 days off therapy and a 6th lumbar puncture was performed (Table 1) The following day bilateral subdural puntures (Table 2) yielded 1 ml of thick fluid from each side Nasopharyngeal blood and fluid from the left subdural tap cultured *Hemophilus influenzae* type B Cultures of cerebrospinal fluid and bilateral middle ear aspirates remained sterile Chloramphenicol was again begun according to the regimen cited previously The patient s sensorium diminished fever persisted above 39 C and by the 28th day he was unresponsive The gravity of the situation was considered and ampicillin added to the therapeutic regimen according to the method described previously On this combined therapy the patient gradually improved and became responsive and afebrile over the next 5 days Chloramphenicol was continued until the 34th and ampicillin until the 37th hospital day A 7th lumbar puncture (Table 1) performed on the 36th day was sterile Bilateral subdural punctures on the 37th day yielded 3 ml of sterile fluid from the left side (Table 2) and none from the right

The patient remained in the hospital for 6 days following the discontinuation of antibiotics During this time he was afebrile and clinically well Neither further diagnostic evaluation nor treatment was thought indicated and he was discharged home on the 43rd day Over the 5 months since discharge he has remained without clinical evidence of infection

DISCUSSION

Various reasons for ampicillin failure in the treatment of *Hemophilus influenzae* meningitis have been suggested Cherry *et al* (2) suggested the inadequacy of the intramuscular and oral routes in achieving bacteriocidal concentrations of ampicillin in the cerebrospinal fluid Gold *et al* (4) experienced relapse when using a dosage of 150 mg of ampicillin per kg per day Greater dosage for certain patients was suggested Coleman *et al* (3) have stressed that the fluctuations of cerebrospinal fluid levels of ampicillin vary directly with the cellular response and indirectly with time

The patient presented in this report was treated intravenously with 309 mg of ampicillin per kg on the first day and 206 mg per kg per day for the succeeding 9 days and then intramuscularly on the 11th and 12 days Tube dilutions of cerebrospinal fluid on the 10th day of treatment demonstrated inhibition of the original organisms at 1 4 These findings suggest adequate antibiotic perfusion of the cerebrospinal fluid Young *et al* (10) suggested that recurrent infection was due to residual focal sequestration of organisms with subsequent reseeding of the blood stream In our patient transient increase of left frontoparietal transillumination and right hemiparesis were

Table 2 *Subdural punctures*

	Hospital day	Side	Vol (ml)	TNC	Gran	Lymph	RBC s	Protein (mg/100 ml)	Culture
1	3	Left	1	98	45	48	800	250	Sterile
2	25	Left	1	—	—	—	—	—	*H infl* type B
2	25	Right	1	—	—	—	—	—	Sterile
3	37	Left	3	3	0	3	0	570	Sterile
3	37	Right	0	—	—	—	—	—	—

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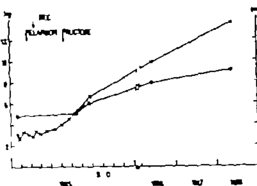


Fig 1 Growth of the patient x——x weight kg o——o height cm

total blood sugar (anthrone method ref 1) were noted In supposedly normal controls small but definite blood glucose increases were obtained on glucose tolerance tests These findings were considered to be in accordance with a diagnosis of GGM or due to an unspecific damage of intestinal mucosa by the diarrhoea producing factor(s) Fructose tolerance test did not give rise to any definite increase of blood fructose or blood glucose (Fructose measured as the difference between total hexose and glucose) In all tolerance tests only small amounts of sugars were recovered in faeces No non absorbable marker substance was used The degree of recovery was therefore unknown making the interpretation of the sugar determinations in the faeces somewhat uncertain

The findings in the fructose tolerance test and in the faeces in all the other tolerance tests were not in all respects as expected in GGM In this condition a definite blood glucose response has earlier been found in fructose tolerance tests (5)

Xylose tolerance test was normal at this age (75 8% of the dose (0 5 g/kg body weight) was excreted in the urine in five hours) as well as at age 16 months (blood xylose 18 and 30 mg/100 ml after 30 and 60 min) At age 7 months pathologically low values were found (blood xylose 6 and 16 mg/100 ml at 30 and 60 min)

Glucose and fructose tolerance tests were repeated at age 7 months when the patient was in good health on her fructose formula Now a definite blood glucose increase occurred when fructose was fed The same was however true also with glucose The diagnosis therefore although practically established by the effect of diet was not settled definitely by the blood sugar assays in the tolerance tests even at this time Within two hours after the glucose feeding diarrhoea occurred which was of course the expected reaction The same reaction had been noted at the earlier tolerance tests with glucose and galactose In the glucose tolerance test 10 g of glucose and 2 g of polyethylene glycol were given 1 3 g of glucose and 0 54 g of polyethylene glycol were recovered in the faeces during 24 hours This indicated that 50% of the glucose dose was excreted in the faeces and glucose absorption was thus very low

Although GGM was now considered the most likely diagnosis it was considered necessary to obtain better biochemical evidence Intubation and perfusion of a segment of the intestine with sugars was therefore performed with a double lumen sond (G Meeuwisse) The proximal lumen reached Treitz ligament and the distal one 25 cm further Through the proximal outlet was pumped at 5 ml/min a solution of 2 2% glucose 2 2 fructose 0 09 NaCl 0 184% urea and 0 2% polyethylene glycol Intestinal contents were sucked continuously through the distal hole After 30 min of equilibration intestinal contents were collected for two periods of 20 min It was found that glucose absorption was 1 9 and 6 7 mg/min/25 cm intestine respectively The corresponding figures for fructose (measured according to Heyrovsky ref 2) were 25 7 and 30 5 The results clearly indicated that fructose was absorbed much better than glucose and definitely proved the diagnosis in our patient

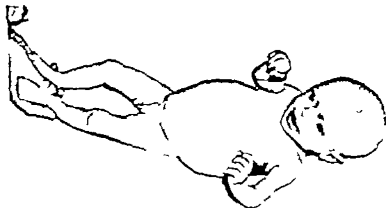


Fig 2 Appearance at age 4 months

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CASE REPORT

DIAGNOSTIC PROBLEMS IN GLUCOSE GALACTOSE MALABSORPTION

A Case Report

K KAIJSER and P A OCKERMAN[1]

From the Department of Paediatrics (Head K Kaijser) Central County Hospital Eskilstuna Sweden

Glucose-galactose malabsorption (GGM) is a rare hereditary disorder of the active absorption of certain sugars including galactose and glucose GGM was first described in 1962 by Lindquist & Meeuwisse (4) and independantly by Laplane *et al* (3) The symptoms and signs are similar to those seen in disacchande malabsorption with diarrhoea as the main problem In the patients published up to now it has been fairly easy to establish the diagnosis as soon as the possibility of GGM has been thought of Simple oral tolerance tests with various mono and disacchandes with assay of sugars in blood and faeces have given a clear and diagnostic pattern (5) In a patient studied by us the findings in such simple tolerance tests did not allow definite diagnostic conclusions For this reason and because only 20 cases have been published earlier including siblings who have died without a diagnosis of GGM but in whom a retrospective diagnosis has been established (6) we want to describe briefly the findings in our patient Our report may illustrate that diagnostic problems do occur in GGM

CASE REPORT

A A a girl was born on January 19 1965 The parents were healthy and unrelated and as far as could be found out not related to the family with GGM described from a different part of Sweden (6) Birth weight 3130 g Immediate neonatal development was uneventful Diarrhoea started on the fourth day and soon became profuse and watery The patient was referred to the Pediatric Department where treatment was started with antibiotics s c and i v infusions of Ringerglucose solutions plasma and bicarbonate Some improvement occurred of the general condition but the patient continued to have diarrhoea Dietary changes with the institution of a lactose free formula did not radically improve the condition of the patient There was nearly no weight gain during the first three months of life Later a slow but steady weight gain occurred All laboratory procedures failed to give a definite diagnosis for reasons discussed below Since GGM was suspected a dietary formula with fructose as the only carbohydrate was instituted at age 4 / months This gave a radical improvement The diarrhoea ceased the general condition of the patient improved and the weight increased On a diet as formulated by Meeuwisse & Melin (5) the development of the patient has since then been normal

Fig 1 outlines the growth of the patient Fig 2 shows the appearance at age 4 months before the institution of the fructose formula

Laboratory tests

Assay of serum electrolytes demonstrated that the patient suffered from hyperosmolar dehydration when the diarrhoea was severe Small amounts of glucose were found in the urine and faeces on several occasions pH in the watery stools was 5 0–5 5 All other routine laboratory tests were normal On glucose galactose and lactose tolerance tests performed during the first months of life only very minute changes of blood glucose (glucose oxidase method commercial kit AB KABI Stockholm) and

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CASE REPORT

PROTEIN LOSING GASTROENTEROPATHY RESPONDING TO CORTICOSTEROID TREATMENT

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From the Department of Pediatrics Beilinson Medical Center Tel Aviv University Medical School Petah Tikva Israel

Gastrointestinal protein loss is associated with many disease entities

Allergic gastroenteropathy as a cause of excessive gastrointestinal protein loss was first described by Waldman *et al* in 1967 (20) The main abnormalities noted were hypoalbuminemia hypogammaglobulinemia anemia eosinophilia edema growth retardation and gastrointestinal protein loss The patients responded well to corticosteroids and to milk free diet

We present here a case with similar features responding to corticosteroid treatment but without response to dietary elimination of milk

Case Report

A 10 year-old Sephardic boy was admitted at the age of 3 years to another hospital with suspected Celiac disease and was found to have protein losing gastroenteropathy with growth retardation There was no family history of diarrhea or edema

At 4 years of age he was first admitted to this department because of diarrhea recurrent vomiting abdominal pain edema and growth retardation His weight and height were in the 50th percentile for age 3 years blood pressure was 90/60 He had generalized edema the liver was palpable 4 cm below the right costal margin Result of the examination was otherwise negative

Laboratory findings Repeated urinalyses were normal The hemoglobin level ranged from 8 8 g/100 ml to 9 6 g/100 ml The white cell count was between 7400–16 100 with a high total eosinophil count of 800– 000 per mm The most remarkable laboratory finding was a very low level of plasma proteins with a total of 2 2 g/100 ml albumin 0 8 g/100 ml globulin 1 4 g/100 ml Quantitative immunoglobulins were as follows IgG 540 mg/100 ml IgM 40 mg/100 ml IgA 52 mg/100 ml The level of ceruloplasmin—0 028–0 100 density units (normal 0 100–0 200) Serum copper was from 23 μg/100 ml to 64 μg/100 ml The serum iron ranged between 33–60 μg/100 ml Serum calcium levels were between 6 8–8 3 mg/100 ml

The following laboratory tests were negative or normal Erythrocyte sedimentation rate platelet counts bleeding and coagulation times prothrombin time fibrinogen protein bound iodine BUN serum electrolytes fasting blood sugar blood total lipids cholesterol phosphates alkaline phosphatase blood bilirubin concentration bromsulfalein retention tests lupus erythematosus cell preparation and Mantoux test

Stool examinations Stools contained no occult blood parasites or pathogenic organisms Repeated D xylose absorption tests and 72 hour stool collections for fat were within normal limits Of an oral dose of I labeled triolein 0 9% was excreted in the stools (normal < 5) Vitamin A loading test showed a rise of serum Vitamin A from 116 to 300 μg/100 ml (normal) Sweat electrolytes were within normal limits Glucose galactose sucrose and lactose absorption tests were normal

On sigmoidoscopy the bowel had a normal appearance An X ray series of the gastrointestinal tract showed a normal esophagus and stomach coarse mucosal folds and rapid flow in the small intestine Barium enema showed no abnormalities A chest roentgenogram was within normal limits X ray for determination of skeletal bone age revealed 4 years retardation

Two per oral jejunal biopsies were taken with a Rubin tube the first at the age of 7 years the second

CONCLUSIONS

The diagnosis of GGM now is definitely established in our patient Our findings with oral tolerance tests illustrate that a diagnosis cannot always be made as easily as in earlier published patients even when the patient does not have diarrhoea Meeuwisse & Melin (5) state that The results of the sugar loading tests and of the dietetic trial with a formula, in which fructose is the only carbohydrate show that during the first months of life a guaranteed diagnosis can be made without further investigations This statement is applicable also to our patient if it is stressed that the dietetic trial was of more definite diagnostic value than the sugar loading tests

REFERENCES

1 Brante G Anthronmetodik for kolhydratbestämningar i det kliniska laboratoriet *Svensk lakartidn* *49* 2516 1952
2 Heyrovsky A A new method for the determination of inulin in plasma and urine *Clin Chim Acta* *1* 470 1956
3 Laplane R Polonovski C Etienne M Debray P Lods J C & Pissarro B L intolerance aux sucres a transfert intestinal actif Ses rapports avec l intolerance au lactose et le syndrome coeliaque *Arch Franc Pediat* *19* 895 1962
4 Lindquist B & Meeuwisse G W Chronic diarrhoea caused by monosaccharide malabsorption *Acta Paediat Scand* *51* 674 1962
5 Meeuwisse G W & Melin K Glucose galactose malabsorption A clinical study of 6 cases *Acta Paediat Scand* Suppl *188* 3 1969
6 Melin K & Meeuwisse G W Glucose galactose malabsorption A genetic study *Acta Paediat Scand* Suppl *188* 19 1969

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from 1 4 to 2 3 g/100 ml (Fig 2) The hemoglobin level increased to 12 2 g/100 ml and the total eosinophil count became normal After 2 months of treatment with Meticorten the daily doses were decreased gradually to 7 5 mg Meticorten per day and he was discharged from the hospital on this daily dosage

Two months later he was readmitted because of reappearance of generalized edema and hypoproteinemia It was found that the child had not received any medication at home during this period He was started on 30 mg Meticorten per day Within 2 weeks the edema had again disappeared The total serum proteins increased from 2 9 to 6 8 g/100 ml Albumin rose from 1 1 to 3 7 g/100 ml globulins from 1 8 to 3 1 g/100 ml This pattern of the recurrence of edema and hypoproteinemia with cessation of the treatment with Meticorten and prompt improvement after reinstitution of therapy was noted several times for the next three years (Fig 2) During those periods when he was not receiving Meticorten several therapeutic trials were attempted with gluten free milk free and milk and gluten free diets Each trial lasted 2–3 months and all were unsuccessful The child remained edematous and hypoproteinemic during these treatments and improved only upon administration of Meticorten (Fig 2)

There has been no improvement in the rate of growth during the 4 years of Meticorten treatment At the age of 10 years he is at the 50th percentile for the age of 5 years

DISCUSSION

The clinical picture the two normal intestinal biopsies the gastrointestinal radiological findings and the normal findings of the sigmoidoscopy led us to exclude the following causes of protein losing enteropathy giant hypertrophy of the gastric rugae (3) ulcerative colitis and regional enteritis (14) Whipple s intestinal lipodystrophy (8) gastrointestinal cancer (7) sprue (9) and constrictive pericarditis (4) A common cause of severe protein loss in infancy and childhood is intestinal lymphangiectasia (6 13 19) In this condition the small intestine histology shows dilated lymphatic channels in the mucosa of the small intestine there is fat malabsorption and there is no response to corticosteroids Since our case presented none of the above phenomena and furthermore showed a clear response to corticosteroids the diagnosis of lymphangiectasia can be excluded

Another common cause in childhood is celiac disease (10 17) In our patient there was no steatorrhea or other evidence of malabsorption the biopsy was normal and there was no response to a gluten free diet We also excluded agammaglobulinemia (6 18) which may be associated with disorders of the gastrointestinal tract with excessive gastrointestinal protein loss

Transient protein loss has also been described with hypocupremic and hypoferremic anemia and eosinophilia (12 15 16) This group appears to represent several entities In our case however the gastrointestinal protein loss is a constant feature and did not change when the anemia had improved

The criteria given by Waldmann *et al* (20) in their report on a number of cases included edema extreme hypoproteinemia anemia eosinophilia growth retardation and gastrointestinal protein loss which was corrected by the administration of corticosteroids and milk free diet They proposed to call this syndrome allergic gastroenteropathy Our case did not respond to several trials with milk free diet and therefore we cannot assume milk allergy to be the cause of the protein loss It does however have features similar to those seen in the cases reported by Waldmann *et al* (20) and it is possible that the cause of the disease is an allergy to an unknown substance

SUMMARY

A 10-year-old boy suffering from generalised edema diarrhea recurrent vomiting abdominal pain and growth retardation was found

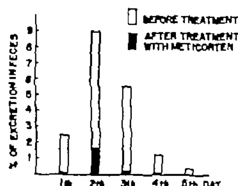


Fig 1 Excretion of [131]I labeled albumin in the gastrointestinal tract before and after treatment with Meticorten (Prednisone)

at the age of 9 /₄ years Both were done when he had been without corticosteroids for at least 2 months and was suffering from generalised edema and diarrhea Both biopsy specimens were of normal appearance

Studies of the protein loss

The loss of plasma proteins into the gastrointestinal tract was estimated by the use of [131]I polyvinylpyrolidone ([131]I PVP) (4 10 17) and [131]I labeled albumin (1 2 13) Each time he was given 12 μCi of the radioactive macromolecule intravenously and the fraction of the injected dose that appeared in the stools passed during the 5 days was determined The methods for the assay of radioactivity in the stools have previously been described (5)

1 [131]I PVP revealed protein loss in stools of 11 7 % of material (normal up to 1 5 % in stools)

2 [131]I labeled albumin with amberlite showed 18 4 % of the material in stools (normal up to 2%) Plasma albumin pool was very low—9 27 g (normal control with similar weight—42 3 g) Albumin half life 1 85 days (normal control 7 7 days) The daily turnover —13 % (normal control —3–4 %)

3 [131]I-labeled albumin test was repeated during corticosteroid treatment and only 2% was excreted in stools (Fig 1)

Therapeutic trials

A high protein diet (4 g per kg) was given for 5 months and was supplemented with 12 5 % salt free albumin and plasma On this regime only a temporary increase was achieved in the serum protein level with no clinical improvement

At this stage 5 months following his admission he was started on 20 mg Meticorten (Prednisone) per day in divided doses Within 3 weeks a marked improvement was noted manifested by disappearance of the edema and diarrhea The total proteins in the serum increased from 2 2 to 4 2 g/100 ml globulins

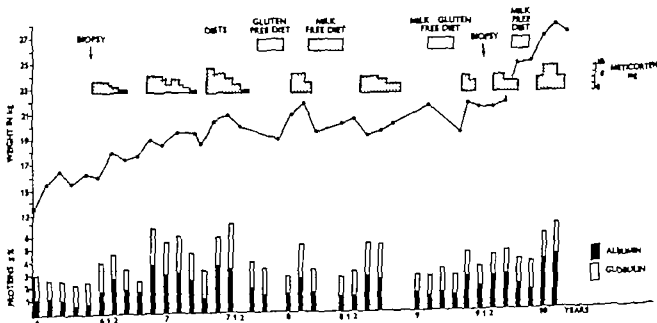


Fig 2 Response of serum proteins to corticosteroids and milk and gluten free diets

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CASE REPORT

EXTENDED HEMODIALYSIS IN AN INFANT

CARL M GRUSHKIN RICHARD N FINE and QUENTIN STILES

From the Renal and Thoracic Surgery Divisions Childrens Hospital of Los Angeles and the Departments of Pediatrics and Surgery University of Southern California School of Medicine Los Angeles California U.S.A

Hemodialysis in infants and small children has been used primarily as treatment for acute renal failure (6 7) and poisoning (5 9) To our knowledge there is only one published report of extended hemodialysis for chronic renal failure in a child less than 3 years of age (10) In addition 2 children between 2 and 3 years of age have been dialyzed for several months while awaiting transplantation (4) The present report describes extended hemodialysis in an 18-month-old infant during a 6 month period while awaiting cadaveric renal transplantation

METHODS

Cannulation The teflon-silastic arteriovenous cannula described by Quinton *et al* (8) was used Under light general anesthesia the superficial femoral artery and saphenous vein were cannulated with no 14 teflon tips

Delivery system A miniature artificial kidney system (MAKS No 900 Bio Systems Inc Santa Monica California) was used to deliver the dialysate solution The delivery system mixed tap water with a commercially prepared solution to the following concentrations calcium 2 4 3 0 mEq l dextrose 200 mg/100 ml magnesium 1 5 mEq l sodium 134 mEq/l potassium 1 or 2 mEq/l acetate 38 mEq/l and chloride 100 mEq/l. This fluid delivery system contains a positive displacement effluent pump which allows negative pressure adjustments from 0 to minus 450 mm Hg to facilitate ultrafiltration

Dialyzer One layer of a specially built / Kiil dialyzer with a total surface area of 0 5 m was used for the first 8 dialyses. Subsequently 1 layer of a standard Kiil was used for all dialyses The fluid volume of 1 layer of a standard Kiil with 8 foot arterial and 7 foot venous blood tubing is approximately 150 ml

Technique of dialysis The blood tubing and dialyzer were primed with 150 ml of 0 9 N saline to which 1500 units of aqueous heparin sodium/1000 ml were added Depending upon the pre-dialysis blood pressure and weight 75 to 150 ml of the priming solution was administered to the patient at the start of dialysis An additional 1000 units of aqueous heparin sodium were infused via the heparin line attached to the arterial blood tubing at the start of each dialysis The clotting time of the venous blood outflow from the dialyzer was monitored every half hour and 500–1000 units of aqueous heparin sodium were administered every 1 to 2 hours to maintain the clotting time between 30 and 60 min

CASE REPORT

M B (B D 3 3 67) a Caucasian female (Fig 1) was admitted to Childrens Hospital of Los Angeles on 7 29 68 at 18 months of age She was the product of a full term pregnancy and uncomplicated delivery Her growth and development were normal At age 15 months a right flank mass was noted The right kidney was not visualized with intravenous pyelography Exploratory laparotomy on 7 6 68 revealed a multicystic right kidney and a right nephrectomy was performed During the procedure the left renal vein was inadvertently interrupted necessitating anastomosis with the inferior vena cava The left renal artery was clamped for approximately 1 / hours during the latter procedure Postoperatively the patient was anuric

She was transferred elsewhere and the clinical diagnosis of acute renal failure due to acute tubular necrosis was made The daily urine output was 60 to 90 ml She was placed on a Franklin diet (3) and fluid intake was restricted to 10 ounces per day Her

to have severe gastrointestinal protein loss with hypoalbuminemia hypogammaglobulinemia and transient anemia and eosinophilia without malabsorption or gastrointestinal structural abnormalities He responded well to corticosteroid treatment but there was no response to gluten free or milk free diet Comparison with similar cases reported in the literature raises the possibility that this is a case of allergic gastroenteropathy due to an undetermined cause

REFERENCES

1 Bauman A & Rothschild M A The rate of intravascular equilibration of intravenously administered I[131] labelled albumin in various body sites *J Lab Clin Med 48* 20 1956
2 Berson S A Schreiber S S & Post J Tracer experiments with I[131] labelled human serum albumin Distribution and degradation studies *J Clin Invest 32* 746 1953
3 Citrin Y Sterling K & Halsted J A Mechanism of hypoproteinemia associated with giant hypertrophy of gastric mucosa *New Eng J Med* 257 906 1957
4 Davidson J D Waldmann T A Goodman D S & Gordon R S Jr Protein losing gastroenteropathy in congestive heart failure *Lancet I* 899 1961
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6 Holman H Nickel W F Jr & Skeneneer M H Hypoproteinemia antedating intestinal lesions and possibly due to excessive serum protein loss into intestine *Am J Med 27* 963 1959
7 Jarnum S & Schwartz M Hypoalbuminemia in gastric carcinoma *Gastroenterology 38* 769 1960
8 Laster L Waldmann T A Fenster L F & Singleton J W Reversible enteric protein loss in Whipple's disease *Gastroenterology 42* 762 1962
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11 Salazar De Sousa J Guerrero O Cunha A & Araujo J Association of nephrotic syndrome with intestinal lymphangiectasia *Arch Dis Child 43* 245 1968
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14 Steinfeld J L Davidson J D Gordon R S Jr & Greene F E Mechanism of hypoproteinemia in patients with regional enteritis and ulcerative colitis *Am J Med 29* 405 1960
15 Sturgeon P & Brubaker C Copper deficiency in infants syndrome characterized by hypocupremia iron deficiency anemia and hypoproteinemia *Am J Dis Child 92* 254 1956
16 Ulstrom R A Smith N J & Heimlich E M Transient dysproteinemia in infants new syndrome I Clinical studies *Am J Dis Child 92* 219 1956
17 Troupel S Acar J Renault H Desbuquois G & Cottan R Enteropathie avec perte de protéines et steatorrhée étude par le PVP I[131] et la trioleine I[131] action du régime sans gluten *Bull Soc Med Hop Paris 76* 261 1960
18 Waldmann T A Steinfeld J L Dutcher T F Davidson J D & Gordon R S Jr Role of gastrointestinal system in idiopathic hypoproteinemia *Gastroenterology 41* 197 1961
19 Waldmann T A & Laster L Abnormalities of albumin metabolism in patients with hypogammaglobulinemia *J Clin Invest 43* 1025 1964
20 Waldmann T A Wochner R D Laster L and Gordon R S Jr Allergic gastroenteropathy a cause of excessive gastrointestinal protein loss *New Eng J Med 276* 761 1967

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Anemia. In general the pre dialysis hematocrit level of all children in our hemodialysis program is maintained at 18% (2). Sedimented red blood cells are given when the pre dialysis level is less than 18%. In a 20 pound infant approximately 20% of the blood volume is circulating in the dialyzer and tubing during dialysis. In order to minimize the effects of a technical mishap the pre-dialysis hematocrit of this patient was maintained at 22%. Approximately 300 ml per month of sedimented red blood cells were necessary to maintain the hematocrit at this level. Several transfusions were necessitated by complications which will be discussed later.

Biochemical. The average pre dialysis BUN, serum potassium and serum calcium are listed in Table 1. Despite pre-dialysis BUN levels of greater than 90 mg/100 ml the infant did not have clinical manifestations of uremia. During the second month of dialysis the pre-dialysis serum potassium occasionally exceeded 7 mEq/l. Avoidance of foods with high potassium content prevented hyperkalemia. Serum calcium levels were maintained within the normal range (8.5–10.5 mg/100 ml) without using supplemental calcium or Vitamin D.

Diet. During the first 1 1/2 months of hemo-dialysis the diet was not restricted. Fluid intake was limited to 12 ounces daily. Fluid retention, hypertension and occasional asymptomatic hyperkalemia (K greater than 7 mEq/l) occurred. Fluid intake was then limited to 8 ounces daily and foods high in potassium, such as milk, potatoes, chocolate and orange juice were eliminated. A calorie count after 4 months of dialysis revealed an intake of 485 calories daily. A new diet was prescribed to increase the caloric intake to 1175 calories daily. However a repeat calorie count after 5 1/2 months of dialysis revealed an intake of only 600 calories daily. Despite this caloric intake, which is less than that recommended, growth occurred (1).

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Weight gain. An indirect assessment of weight gain is shown in Fig. 2. Comparison of pre-dialysis weight versus pre-dialysis blood pressure shows that a direct relationship exists between fluid retention and increased blood pressure. Fluid removal during dialysis was followed by a decrease in blood pressure. During the November–March period of dialyses the post-dialysis weight increased from 18 to 20 pounds without alteration of the post-dialysis blood pressure. These observations suggest that the weight gain is "real" rather than a reflection of retained fluid.

Complications. Superficial skin infection which responded rapidly to oral antistaphylococcal antibiotics occurred on one occasion around the arterial exit site of the cannula

Table 1. *Pre dialysis average value per month*

	BUN (mg%)	Serum potassium (mEq/l)	Serum calcium (mg%)
October	25	4.1	7.6
November	51	5.9	9.6
December	63	4.9	9.1
January	112	6.1	9.4
February	94	5.2	8.4
March	75	5.0	8.5



Fig 1 Patient after 5 months of dialysis

condition failed to improve and between July 1 and September 20 1968 7 peritoneal dialyses were performed Between dialyses she was maintained on a low potassium Franklin diet supplemental calcium vitamin D Amphojel® and limited fluid intake She was transferred to Childrens Hospital of Los Angeles for hemodialysis and renal transplantation

Physical examination upon admission revealed a debilitated infant with decreased muscle mass and subcutaneous tissue Moderate edema of the face hands and feet was present There were no signs of vitamin deficiency or bleeding The blood pressure in the recumbent position was 130/90 mm Hg the pulse rate was 118 per minute and respiratory rate 30 per minute The height was 29 inches and weight 19 and / pounds There was a right nephrectomy scar and several small midline scars over the abdomen The liver was not enlarged and there were no palpable abdominal masses Radiographic examination of the chest showed infiltrates in the right middle and upper lobes The heart size was normal There was no radiographic evidence of rickets

On admission the hemoglobin was 7 9 g/100 ml platelets 47 000/mm blood urea nitrogen (BUN) 48 mg/100 ml serum creatinine 4 6 mg/100 ml serum sodium 150 mEq/l serum potassium 4 1 mEq/l serum calcium 7 5 mg/100 ml and serum albumin 2 8 g/100 ml

A teflon silastic arteriovenous cannula was inserted on 23 9 68 and hemodialysis was begun on 24 9 68 Because of persistent vomiting an upper gastrointestinal series was obtained on 27 9 68 No barium was seen beyond the duodenum indicating a probable high small bowel obstruction While preparing for surgical exploration a cadaveric kidney from a 48 hour old anencephalic male newborn became available A renal transplant was performed and adhesions involving the small bowel were lysed Within 30 min after completion of the vascular anastomosis the transplanted kidney became progressively darker in color A diagnosis of hyperacute rejection was made and the kidney removed On 11 10 68 a second laparotomy was performed because of persistent vomiting and fresh adhesions involving the small bowel were lysed No further vomiting occurred On 17 10 68 she was discharged without medications to return thrice weekly for hemodialysis

RESULTS AND COMMENTS

Arteriovenous cannula The superficial femoral artery and saphenous vein were of sufficient caliber to insert no 14 teflon tips With tips of this size, flow rates greater than 100 ml/min were obtained without the aid of a blood pump provided that systolic blood pressure was greater than 90 mm Hg Williams described arteriovenous cannulation utilizing an artery and vein in the forearm of a 2 1/ year old child (10) Flow rates through the specially built dialyzer averaged 40 ml/min Fluid removal with this low flow rate was difficult and 2 peritoneal dialyses were necessary during a 6 week period to reduce edema

Blood pressure Monthly average pre and post dialysis blood pressures are shown in the top half of Fig 2 Systolic blood pressures

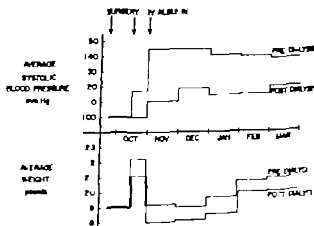


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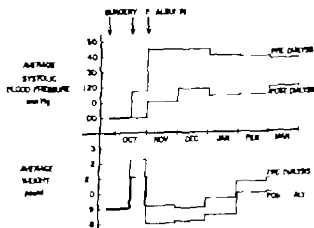


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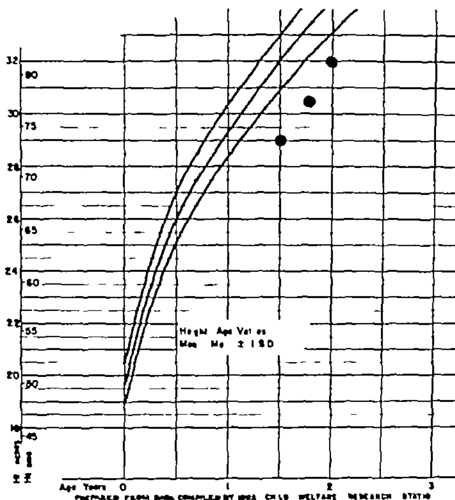


Fig 3 Growth chart

Clotting in the arteriovenous cannula occurred on 4 occasions The clot was easily removed on the first 2 occasions The third necessitated surgical revision with a new teflon tip placed in the artery and the fourth necessitated transfer of the cannula to the other leg The initial cannula functioned for 5 ¹/ months Blood transfusion to raise the hematocrit to 30 % was necessary to prepare the patient for both surgical procedures Clotting of the dialyzer occurred once and a transfusion was needed for replacement

During three dialyses the systolic blood pressure fell rapidly to 60–70 mm Hg due to excessive ultrafiltration and fluid removal Hypotension was easily and rapidly corrected by the administration of 0 9 *N* saline via the venous drip chamber

Clinical response On admission to the hospital the infant was hypotonic, did not sit without support or smile After 2 months of dialysis she sat without support and after 4 months walked with support Her affect improved and she began to smile and talk after 3–4 months of dialysis

SUMMARY

Extended hemodialysis thrice weekly for 6 hour periods in an 18 month old infant with end stage renal disease reversed the clinical manifestations of uremia A single layer of a standard Kiil dialyzer was used Cannulation of the superficial femoral artery and saphenous vein with no 14 teflon tips permitted flow rates through the dialyzer of greater than 100 ml/min without the aid of a blood pump Ultrafiltration during dialysis permitted fluid removal and alleviated hypertension Cannulation of large vessels obviated repeated clotting

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Addendum On 26 3 69 the infant received a cadaver renal homotransplant At the present time 5 69) the child is doing well and renal function adequate

REFERENCES

1 Church C F & de Planter Bowes A Food values of portions commonly used J B Lippincott Company Philadelphia 1966 10th edition

2 Fine R N Korsch B M Grushkin C M & Lieberman E Hemodialysis in children Submitted for publication 1969

3 Franklin S S Gordon A Kleeman C R & Maxwell M H Use of a balanced low protein diet in chronic renal failure JAMA 202 477 1967

4 Holliday M Personal communication

5 Kallen R J Zaltzman S Coe F L & Metcoff J Hemodialysis in children technique kinetic aspects related to varying body size and application to salicylate intoxication acute renal failure and some other disorders Medicine 45 1 1966

6 Lee H A & Sharpstone P Hemodialysis in pediatrics Acta Paediat Scand 55 529 1966

7 Moorhead J F Edwards E C & Goldsmith, H J Haemodialysis of three children and one infant with a haemolytic uraemic syndrome Lancet I 570 1965

8 Quinton W Dillard D & Scribner B H Cannulation of blood vessels for prolonged hemodialysis Trans Amer Soc Artif Intern Organs 6 104 1960

9 Spritz, N Fahey T J Jr Thompson D D & Rubin A L The use of extracorporeal hemodialysis in the treatment of salicylate intoxication in a 2 year-old child Pediatrics 24 540 1959

10 Williams A V Hargest T S & Wohltmann H J Chronic hemodialysis of a 2 year-old child Pediatrics 43 116 1969

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Key words Haemodialysis infant

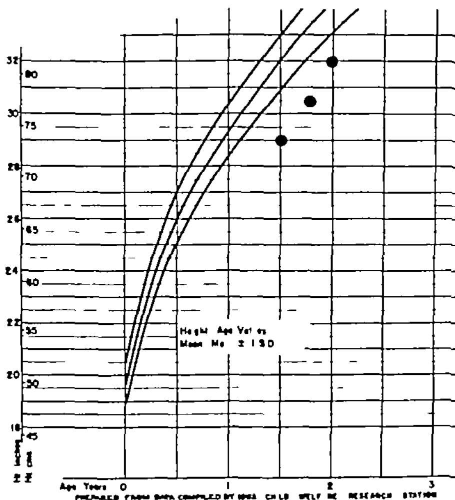


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F Holstad *Staphylococcus aureus infections in the newborn*

J Ek *Staphylococcus aureus osteomyelitis in the neonatal period*

During the period 1956–67 altogether 111 cases of acute hematogenous osteomyelitis were seen in these paediatric wards The disease occurred relatively more frequently in the neonatal period than in any later age period as many as 16 aquired the disease in the first month of life and in 13 of these cases the etiological agent was proved to be staphylococcus aureus An accumulation was found in the period 1958–62 when staphylococcus aureus infections were a serious problem in maternity hospitals

Multiple bone affections were seen in 4 out of the 13 neonates with staphylococcus aureus infection Three had maxillitis Local swelling and leukocyte reaction were the most constant positive findings on admission most patients had normal temperature and ESR and only moderately affected general condition Five patients had pathological X ray findings on admission, the others developed this later in the course or the diagnosis was made from material from the infectious focus Blood cultures gave little diagnostic information in the material

The prognosis was more serious in the neonatal period compared with infancy or early childhood 1 died 3 developed sequelae and 1 of these chronic osteomyelitis

T Midtvedt *Toxoplasmosis*

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A newborn infant with Inclusion Body Disease was described The infant was asphyxiated at birth and remained cyanotic with respiratory distress throughout the course

Icterus developed at the 2nd day of life There was no hepatosplenomegaly Eye grounds were normal The infant was treated for hypoglycemia convulsions and sepsis He matemesis and heart failure developed at 33 days of age when the infant died Three examinations for cytomegalic inclusion bodies in urine were negative Postmortal examination showed inclusion bodies in kidneys thyroid pancreas and lungs

G Fluge V Lehmann & P J Moe *Hereditary fructoseintolerance*

R Lindemann *Tyrosine metabolism in fructosaemia*

The acute form of fructosemia in early infancy may show the same clinical picture and the laboratory findings as acute tyrosinosis Both types of patients may have hepatomegaly edema ascites pathological liver function tests proteinuria mellituria generalized aminoaciduria and striking excretion of phenolic acids

The levels of the serum amino acids are elevated both in fructosemia and "acute tyrosinosis but in fructosemia there appears to be a more generalized aminoacidemia Methionine is very high in both disorders in the acute stage and tyrosine is elevated particulary in acute tyrosinosis A marked generalized aminoaciduria is found in both disorders The excretion of proline was particularly high in fructosaemia and only moderately increased in "acute tyrosinosis A marked excretion of the phenolic acids is seen in both types of patients In the cases with fructosemia the tyrosyluria disappeared as soon as fructose was removed from the diet It may be concluded that it is not possible to make a differential diagnosis between fructosemia and "acute tyrosinosis on the basis of the urine and serum amino acids and the phenolic acid excretion when the child is on a normal diet

Acta Pædiat Scand 59 226-230 1970

PROCEEDINGS OF PAEDIATRIC SOCIETIES

NORWEGIAN PAEDIATRIC SOCIETY

Meeting June 12-14 1969

Infections in the neonatal period

O J Mellbye *Immunological conditions in the newborn*

O Garborg *Neonatal purulent meningitis*

Summary 10 cases of neonatal purulent meningitis were treated at the Children s department Sentralsykehuset i Trondheim from April 1 1963 to April 1 1969 Infants with obvious congenital defects of the central nervous system were excluded

Three cases were due to *E coli* 1 to *Aerobacter aerog* 1 to *Proteus mirabilis* 1 to *Staphylococcus aureus* 2 to Group B Streptococci and 1 to *Listeria monocytogenes* In one case no organism could be cultured from the cerebrospinal fluid

The characteristic features of meningitis in the newborn period were discussed The importance of Group B streptococci the high vaginal carrier rate among pregnant women and the cross infections seen in maternity units were emphasized

The prognosis is poor even with modern therapy The mortality rate is 50-60% with a high frequency of sequelae in the survivors In the present series 2 infants died 3 developed hydrocephalus At a follow up 1 to 5 years after the acute meningitis 2 patients were apparently without sequelae 3 were moderately and 3 severely handicapped (mental retardation convulsions spasticity deafness squint)

Recent studies suggest that maternal carriers of certain bacteria (Group B Streptococci *Listeria monocytogenes Vibrio fetus*) may comprise a high risk group The eradication of these organisms from the genital tract during pregnancy may be of great prophylactic value

J Marstrander *E coli meningitis*

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In the period April 63-Dec 68 437 cases of pneumonia were registered at the Department of Pediatrics Sentralsykehuset Trondheim 39 occured during the neonatal epriod 24 boys and 15 girls The initial symptoms were in half of the patients noted during the first two days of life The most common symptoms were cyanosis (13) nasal congestion (12) coughing (9) and apnoeic spells (4) Four infants were febrile 17 patients were premature Abnormal pregnancy was noted in 11 cases and pathological delivery was registered in 15 mothers Relatively often the infants were asphyxiated after delivery The blood picture was uncharacteristic and bacteriological cultures from nose and throat were of little value for the therapy In 4 patients the chest X ray revealed distinct pulmonary infiltrates whereas the changes were uncertain in 27 cases Nine infants were treated with penicillin and streptomycin 8 with ampicillin and 11 with cephaloridine The remaining group was treated with other combinations of antibiotics One patient with staphylococcal pneumonia received methicillin and fucidin 26 patients recovered in a few days 10 showed a protracted course of

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	No tested		Au positive		Antigen?
	Children	Adults	Children	Adults	No
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Acute myeloblastic	5	0	1	0	
Chronic lymphocytic	0	3	0	1	
Other types	1	1	0	0	
Hodgkin s Disease	3	6	1	1	(1)
Lymphosarcoma	2	0	0	0	
Reticuloendotheliosis	7	0	0	0	
Solid tumor	9	0	0	0	
Osteop trosis	2	0	0	0	
Various other blood dis	20	0	0	0	(1)
Total	84	10	5	2	(3)

rum from a child with acute leukemia This child had not received any blood transfusion or hepatotoxic drugs

A new precipitating antigen antibody system (Pennsylvania) has recently been discovered Pennsylvania antigen seems to occur in about the same diseases as Au antigen

J Steen *Syndroma Kallmann sive de Morsier*

This syndrom is rare Mostly boys are affected and they have pituitary hypogonadism and anosmia

The patient demonstrated is born in 1949 He has been under observation the last 5 years due to deficient puberal development No known relatives with the same syndrom The patient has never had any serious diseases or injuries normal psychic development slow growth of height from the age of eleven he is now 168 cm tall Approximately normal proportions of the body no gynecomastia Infantile testis about 1 2 cm both descended

Apart from scarce pubic hair no secondary sexual characteristics Normal PBI normal ACTH secretion both by Metopirontest and in direct serum tests Very low or lacking gonadotropin in repeated tests Testosterone in plasma subnormal Sex chromatin negativ He has a total anosmia

The only possible differential diagnosis is pubertas tarda The patient has for the last year been treated with human gonadotropin in cooperation with Pediatric Research Institute Rikshospitalet Oslo Despite the fact that there has been an increase of testosterone in plasma up to lower normal values there can still not be observed any clinical effect of the treatment

K H Torp *Menarche in North Norway*

J Steen Johnsen *Coma subsequent to measles vaccination*

Encephalitis is reported to occur in 1–4 of measles patients More than 50 of children with uncomplicated measles present abnormal EEG The last years the central nervous disease SSPE (subacute sclerosing panencephalitis) has been related to measles Years following the acute disease the cerebral degeneration occur presenting with increased measles antibody and living virus in the nervous tissue

Living measles virus vaccine has recently been suspected to cause encephalitis in England (Beckenham 20 strain) A more attenuated strain is used in Norway (Schwartz) From USA 23 possible encephalitis cases were reported in 14 8 million inoculations with living vaccine a rate far below the spontaneous measles encephalitis

The possibility of measles vaccine virus

It is therefore of importance to exclude fructosaemia in cases presenting the clinical picture of acute tyrosinosis in infancy

H Pande & E Ruud *Lowe s syndrome*

Lowe s syndrome is a hereditary disease in the group inborn errors of metabolism associated with aminoaciduria first described in 1952 The basic biochemical defect is unknown It is characterized by the following main symptoms bilateral congenital cataract and/or glaucoma, psychomotor retardation, retarded growth generalized hyperaminoaciduria proteinuria metabolic acidosis rickets hypotonia and in some cases glycosuria The affected child has a characteristic look Two brothers suffering from Lowe s syndrome were reported They both had findings typical of the disease The elder one had rickets which responded well to 460 000 units of vitamin D for a course of nine days A brief discussion of the different symptoms were given

K B Cyvin *Two siblings with Lowe s syndrome*

Two siblings, a boy and a girl with the characteristic features of the oculo cerebro renal syndrome of Lowe are described In both cases the impaired renal function was a predominant feature of the disease and both died at the age of 3–4 months

J Weidemann *Oftalmological findings in Lowe s syndrome*

T Feyling *Subcutaneous fat necrosis with hypercalcemia in infants*

A case of neonatal subcutaneous fat necrosis with evidence of hypercalcemia was described The eight cases from the literature were briefly discussed

The serum calcium levels decreased to normal after administration of prednisone and limitation of dietary vitamin D and calcium

No complications are apparent after 10 months

Malignant diseases in children

E Bjerke *The frequency of malignant diseas in children in Norway*

O Knutrud *Diagnosis and treatment of malignant tumors*

P J Moe *Treatment of malignant diseas with cytostatics*

M Seip *Treatment of acute leukemia*

P J Moe *Antigen-antibodies in malignant blood diseases*

The Australia precipitating antigen antibod system was discovered by Blumberger *et al* i 1964 Australia (Au) antigen is demonstrat by a double diffusion technique using a micr Ouchterlony technique The antigen may al be seen with an electron microscope afte ultracentrifugation on a sucrose gradient It about the same size as has been postulated fo hepatitis virus and it is possible that Au an tigen is the same type of virus

Au antigen occurs extremely rarely i American and Scandinavian population Blumberger and co workers have recentl shown that Au antigen occurs in about 10 15% of cases of acute myelogenous leukemia chronic lymphatic leukemia, acute lymphati leukemia and Hodgkin s disease and in abou 30% of institutionalized patients with Down syndrome Au antigen also seems to be a tem porary finding in serum from about 20% o patients with hepatitis

A preliminary report is given of the result of Au antigen studies performed during the las year on serum from Norwegian children (and a few adults) The serum studies were per formed by Dr L Melartin, Helsinki The inci dence of Au antigen in leukemia and Hodgkin s disease in childhood (Table 1) was found to be about the same as stated above Negative reactions were found in sera from 40 children with other malignant diseases or other blood disorders except for a question able positive test (positive reaction to human antiserum and negative to rabbit antiserum) in a child with congenital hypoplastic throm bocytopenia Anti Au was demonstrated in se

NEW BOOKS RECEIVED

L. P Hollander & M Matthes (eds) *Ergebnisse der Bluttransfusionsforschung* Vol X Bibliotheca Haematologica No 32 348 pp illus S Karger AG Basel New York 1969 sFr 79 US $18 95

N Lloyd Rusby (ed) *New ideas in asthma and its management* 56 pp The Chest and Heart Association Health Horizon Ltd London 1969 10s

J Vialatte *L'enfant allergique* 164 pp Expansion Scientifique Française Paris 1969 32 F

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U Brunner *Das Lymphödem der unteren Extremitäten* 248 pp illus Verlag Hans Huber Bern 1969 DM 44

P G Kiss & L P Liebermann *Persönlichkeitsstörungen im Kindesalter* 398 pp illus Akadémiai Kiadó Budapest 1969 US $24 00

G E W Wolstenholme & M O'Connor (eds) *Foetal autonomy* A Ciba Foundation Symposium 326 pp illus J & A Churchill Ltd London 1969 70s

L Moor *Lexique Français Anglais Allemand des termes usuels en psychiatrie neuro-psychiatrie infantile et psychologie pathologique* 224 pp Expansion Scientifique Française Paris 1969 24 F

P Gardiner R MacKeith & V Smith (eds) *Aspects of developmental and paediatric ophthalmology* Clinics in Developmental Medicine 32 126 pp illus W Heinemann Medical Books Ltd London 1969 42 s

L Diethelm O Olsson F Strnad H Vieten & A Zuppinger (eds) *Handbuch der medizinischen Radiologie* Vol X Teil 1 *Röntgendiagnostik des Herzens und der Gefässe* (H Vieten (ed)) 789 pp illus Springer Verlag Berlin Heidelberg New York 1969 DM 296 US $74 00

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Ch M Poser (ed) *Mental retardation Diagnosis and treatment* 284 pp illus Hoeber Medical Division Harper & Row Publ New York 1969 US $9 50

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P Lichtlen *Klinische Vektor-Elektrokardiographie* 235 pp illus Springer Verlag Berlin Heidelberg New York 1969 DM 86

causing SSPE is suspected but not prooved A 2 year old girl was admitted to the Childrens Department Porsgrunn one week after measles vaccination she was in coma with clinical and EEG signs of encephalitis A strong curry-like smell was later on recognized She had since 9 months of age suffered some episodes with febrile convulsions Diagnostic virus investigations revealed no growth of measles virus and the antibody response was very weak Aminoacid chromatography in urine and blood showed great excess of leucin, isoleucine and valin Following 10 days treatment with low protein diet the girl was in clinical and biochemical normal condition She is not mentally retarded

Seven children is previously known with this late manifesting variant of Maple Syrup Urine Disease, 3 of these from Norway (dr R Kal) The measles vaccination with viremia has probably precipitated the further derangement in this inborn enzymatic error of metabolism and no real encephalitis was proved

Children with previous convulsions should not be given measles vaccine

O Garborg *Nutrition problems in East Africa*

Thor Oistein Endsjo

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BOOK REVIEWS

Charles Varga *Handbook of pediatric medical emergencies* The C V Mosby Co St Louis Missouri 1968 694 pp $19 75

The success of the future generation lies dormant in this child Therefore in this hour the future of tomorrow lies in your hands How then will you shape his destiny and that of the world? Yours is an awesome responsibility Fulfill that responsibility as if your very life depended upon it

The demand expressed in this quotation from J C Edwards one of the 14 contributors to the book is really an urgent one There is great need for good advice in medical emergencies The 4th edition of this handbook discusses almost all pediatric medical emergencies including cardiovascular neurologic psychiatric respiratory endocrine hematologic ophthalmologic genitourinary and metabolic emergencies There are also articles on emergencies in the newborn on emergencies due to physical and chemical agents and due to hypersensitivities and toxins It is not a task for a general paediatrician to value the facts given by different experts but as far as I can find these facts are up to-date and in accordance with routine treatment at a pediatric clinic A little too routinary perhaps The greatest advantage of this handbook is the surveyable arrangement of the text One can rapidly find the facts elucidating the acute situation Therapeutic doses of drugs are arranged in tables The doses are correlated to body weight or surface area throughout the book and there are many short useful notes and comments About 90 pages of the book deal with the problems of poisoning Here I miss information on toxic doses In Sweden we have had experiences of the good effect of coppersulphate as an emetic In this book copper sulphate is mentioned only as a poison One of the most informing chapters deals with pediatric procedures There are many illustrations and most of them are very good There is however one illustration which in my opinion is somewhat misleading showing superficial temporal veins suitable for scalp vein puncture My experience is that those vessels are very fragile and inferior to those on the forehead There are about 1000 references This handbook of pediatric medical emergencies can be recommended to be read by anyone who may get in such a situation and ought to be available for rapid consultation in every emergency room Thereby it can surely make the doctor s awesome responsibility a little bit less awesome

Bo Balldin

R Mac Keith & M Bax (eds) *Studies in infancy* Clinics in Developmental Medicine No 27 W Heinemann Medical Books Ltd London 1968 109 pp 25s

This booklet is mainly based on works dealing especially with neonatal neurology presented at the meeting of the 5th International Study Group at Oxford in 1966

In 40 pages Prechtl and his coworkers describe their extensive polygraphic studies on full term newborns The standard variables recorded were respiration EKG EEG EMG and eye movements Some papers are dealing with abnormal neurological conditions e g convulsions from endocrinological point of view and characteristics of fits in the newborn period The relationship between perinatal incidence and later development of the children is discussed in five papers In the reviewer s opinion this is the most valuable part of the book emphasizing not only the value of detailed studies on a newborn infant but also the difficulty in drawing prognostical conclusions from the findings of these studies

The value of such a book is that the reader gets in a condensed form the experiences from various groups in the world on some special problems

Tor Lindberg

J Brodehl *Der renale Transport der Aminosäuren im Säuglings und Kindesalter* Ferdinand Enke Verlag Stuttgart 1969 97 pp DM 30 —

Unter Verwendung von automatisierter Säulenchromatographie bestimmte der Verf die freien Aminosäuren im Serum und Urin sowie den Inulin und PAH Clearance unter Standardbedingungen bei Säuglingen und Kindern Der Verf gibt seine Resultate in dieser sehr sorgfältig durchgeführten Untersuchung über den renalen Aminosäuretransport wieder Er diskutiert seine Resultate mit den bisher nur im geringen Umfang vorliegenden Resultaten anderer Untersucher und schliesst damit eine wichtige Lücke Die Resultate des Verf können somit als gute Referenzbasis für andere Untersucher bei ihren klinischen Studien über Aminosäuretransport Probleme dienen

H v Studnitz

Acta Pædiat Scand 59 233–240 1970

THE SIGNIFICANCE OF THE DEFICIENCY STATE IN LESCH–NYHAN DISEASE

H GHADIMI C K BHALLA and D M KIRCHENBAUM

From the Department of Pediatrics Methodist Hospital of Brooklyn and the Department of Biochemistry State University of New York New York U S A

X linked hyperuricemia associated with destructive finger and lip biting, choreoathetoid movements spasticity and mental retardation was first recognized as an entity distinct and different from adult gout by Lesch & Nyhan in 1964 (5) Since the most obvious and widely recognized biochemical finding is increased concentration of uric acid in the blood and urine conventional therapy in all twenty two cases so far described has been aimed at reduction of the uric acid level in blood

Perhaps by analogy with adult gout reduction of blood uric acid has been attempted by institution of a low purine low protein diet Contrary to this opinion we believe that institution of a high protein diet and provision of purine precursors is essential to the treatment of these patients

TECHNIQUE

Blood samples for amino acid analysis were taken in dry heparinized tubes and centrifuged to obtain the plasma This was then deproteinized by adding 1 picric acid (1 4 V V) The mixture was centrifuged and the supernatant removed Assuming that packed picrate precipitate contains the same concentration of free amino acids as the supernatant fraction 5 ml of supernatant represent an aliquot of 1 ml of plasma Twenty four hour urine samples were collected in bottles containing a small crystal of thymol and kept refrigerated. The volume was measured and a small amount filtered A 5 ml aliquot of filtered urine was deproteinized by the addition of 1% picric acid (1+4 V/V)

An aliquot of the picrate supernatant representing 1 ml of plasma or urine was pipetted into a column of Dowex 2 X8 (ionic form Cl) to remove the picrate The column was washed six times with 2 ml portions of 0 01 N HCl and the combined effluent evaporated to dryness on a rotary evaporator The temperature of the water bath did not exceed 40 C The dry samples prepared in this manner were stored in the deep freeze Since it has been our experience that the glutamine in samples is unstable even in the dry state in the deep freeze blood samples were analyzed by column chromatography on the day they were drawn Two cerebrospinal fluid samples were obtained and treated in the same way

Free amino acids were measured by the use of an amino acid analyzer built according to the Piez modification (11) of the technique originally described by Spackman *et al* (14) In our previous studies on cerebrospinal fluid it became mandatory to separate glutamine from adjacent peaks This was eventually achieved by adjustment of the column temperature Two tenths µmole of norleucine was added to the samples before application to the column as an internal standard for checking the color yield Judged by the recovery of this substance the reproducibility is ± 4 7

Uric acid was determined by the use of an automatic analyzer based on the method described by Newton (7) Three tenths ml of diluted urine (1 10) and 0 3 ml of serum were applied to the analyzer Based on application of 10 mg uric acid as an internal standard the reproducibility of the technique is ± 3 0%

The method used for hypoxanthine guanine phosphoribosyl transferase (HG PRTase) activity determination by Dr Nyhan s laboratory is a modification of that described by Kornberg *et al* (4)

This study was supported by Research Contract 1 RO 1 HD 0399 -01 from the National Institute of Child Health and Human Development.



Fig 1 Patient prior to treatment Note spasticity and strong urge to bite fingers

CASE REPORT

Patient A S was the product of an uneventful full term pregnancy low forceps delivery weighing five pounds twelve ounces at birth The parents were not related The maternal great grandmother and great great grandfather had gout A male first cousin of the maternal grandmother died at eight months because of continuous illness Otherwise the clinical family history is non-contributory The patient s neonatal period was reported uneventful except that each feeding took a long time No developmental abnormality was noticed by the parents until the baby was six months old It was then observed that the infant could not sit even with support

At one year of age his head circumference was 40 5 cm his height was 70 cm (below 3rd percentile) and he weighed 9 5 kg (25th percentile) He could roll over lift his head lean forward on his hands and reach out to grasp large objects (with some tremors) He attempted to receive dropped objects and responded to his name played peek a boo and repeated some consonant sounds e g cat Ma He could not sit without support transfer objects from one hand to another pull himself to a standing position or grasp objects with his thumb and forefinger He cried when he was hungry

On physical examination at one year of age except for hypotonia physical retardation and small stature no other abnormality was noticed Reflexes were within normal limits His plasma and urine free amino acids examined by column chromatography were within the normal range Blood uric acid was determined although the patient had no history of finger biting or spasticity The value was 5 2 mg/100 ml The parents were advised to bring the child after six months for re-evaluation

The patient returned at 3 ¹/ years of age revealing a history of finger biting which started at two years spasticity and increasing irritability His blood uric acid at this time was 8 8 m /100 ml

The patient was admitted with the diagnosis of Lesch Nyhan Disease Scrutiny of the dietary history revealed that his diet generally consisted of the following *Breakfast*— /₂ to 1 slice of toast / cup or less of cereal and liter of juice *Lunch*—1 cup of squash or spinach / cup macaroni one piece of cake for dessert and ¹/ liter juice *Snack*—several cookies *Dinner*— / cup vegetables potato 10-15 g meat and / liter juice Based on the above items the daily intake was less than 1000 Calories and less than 10 g protein per day

Physical examination on admission revealed a hyperirritable poorly nourished and poorly developed child His weight was 9 9 kg and his height was 76 cm both below the third percentile He constantly said juice asking for a drink He screamed when approached and went into opisthotonus His face had a vacant look His arms were splinted and his hands were gloved On removal of the elbow splints and gloves his fingers *immediately* went to his mouth Hard biting and bleeding followed His action in this respect was similar to an addict craving drugs Raw wounds from previous bitings were observed on all the finger tips He did not bite his lips (Fig 1)

Neurological consultation records a hopelessly retarded child His speech was not distinct and he was staring in space his back and neck were constantly in opisthotonus He showed marked hyperirritability and spasticity The child kept his legs in a scissoring position Deep tendon reflexes were brisk Plantar responses were extensor Ankle clonus was sustained Response to painful stimuli was normal Examination of other systems revealed no abnormality

Routine analysis of blood urine and CSF as well as EEG and skull X ray did not show any abnormal result X ray of the wrist suggested a bone age of approximately 2 years

INVESTIGATION

Preliminary investigation during the first two weeks of hospitalization consisted of 1) close clinical observation of the patient s condition with particular emphasis on his diet 2) repeated analysis of the patient s blood urine and cerebrospinal fluid for amino acids and uric acid 3) chromosomal and enzymic studies of the patient s blood No amount of persua

Table 1 *Serum and urinary uric acid values of the patient and his immediate relatives compared to controls*

Patient s serum uric acid obtained at one year not included

Subject	Age (years)	Serum uric acid (mg/100 ml)	Urinary uric acid mg/'4 hrs	Urinary uric acid mg/g creatinine
Patient	3½	8 8–15 0	369 0	3719 0
Sister	1½	4 3	148 2	200 0
Father	28	5 4	704 5	393 3
Mother	26	5 9		
Maternal grandfather	59	5 2	393 0	545 4
Maternal grandmother	47	4 1	187 0	474 1
Maternal uncle (M)	18	5 5	521 4 1372 4	448 7–4594 0
Maternal uncle (J)	23	5 8–6 2		
C ntrols for serum				
12 Children (7 M 5 F)	3–6	3 6 (2 5–5 9)		
23 Male adults		3 –7 1		
1 Female adults		2 6–5 2		
C ntrols for urine				
11 Children (3 M 8 F)	5 mos –6 yrs		62 03 (1 8–291 5)	761 0 (175 0–2166 6)
7 Male adults			527 0 (440 0–710 0)	342 0 (243 0–406 0)

Before treatment
Based on determinations
Based on 3 determinations

sion could induce the patient to accept any high protein food including raw or cooked hamburger bloodwurst etc The results of the blood and urinary uric acid determination of the patient and several of his close relatives are presented in Table 1 Chromosomal studies were normal

Two weeks after hospitalization allopurinol therapy (150 mg/day) was started The response of the blood uric acid level was dramatic within five days it was reduced to 2 5 mg 100 ml

Excessive formation and excretion of uric acid which requires glutamine glycine and aspartic acid in the face of prolonged low protein intake indicated to us the necessity for administration of a high protein diet in association with allopurinol therapy After 14 days of allopurinol therapy the patient still could not be persuaded to take the prescribed amount of protein Studies of free amino acids of the blood samples taken at one year of age and during the first four weeks of hospitalization showed persistently low glutamine concentrations (8 4 4 4 and 2 5 mg/100 ml — See Table 2) Since monosodium glutamate (MSG) is commercially available for the purpose of making food more appetizing it was added to the diet in the amount of 10 g/day The patient liked the taste and his appetite steadily improved

Six weeks after admission that is two weeks following the administration of MSG his daily intake rose to 38–70 g protein per day and 1000–1500 Calories per day At this time there was a definite change in his disposition as judged by the House Staff members nurses and neurologist He became a smiling happy "hello-good by" child with a "winning personality" as the neurologist later recorded His legs were relaxed while in a supine position While holding him in a standing position he imitated walking His scissoring and stiffness decreased and opisthotonus disappeared He started to answer simple questions such as "How are you?" and "Where is Mommy?" He could sit in his walker and cruise about using his toes most of the time The urge to

Table 2 *Free amino acid concentrations of the patient s plasma before and after the administration of monosodium glutamate (MSG) as compared to control values*

Three samples were obtained prior to MSG therapy 16 samples were obtained while on treatment

	Plasma free amino acids before MSG Mean Range	Plasma free amino acids from 10 child controls Mean Range	Plasma free amino acids after MSG Mean Range
Glutamine	5 1 2 5–8 4	12 1 5 9–17 1	9 8 6 1–18 6
Glutamic acid	0 9 0 6–1 2	1 2 T–3 3	1 7 0 5–7 1
Glycine	1 1 0 7–1 3	1 1 0 8–1 4	1 7 0 5–2 9
Aspartic acid	0 2 0 1–0 4	0 1 T–0 2	0 2 0–0 5
Threonine	1 1 0 2–1 8	1 4 0 8–1 8	1 9 0 7–3 5
Serine	1 4 0 7–2 2	1 1 0 6–1 7	1 8 0 7–3 9
Proline	2 3 1 6–3 3	1 9 0 8–3 3	2 8 1 4–5 6
Alanine	3 7 1 9–5 5	2 3 1 4–3 8	4 4 1 7–8 5
Cystine	0 8 0 03–2 2	0 5 0–0 8	1 1 0–2 2
Methionine	0 3 0 1–0 6	0 2 0 02–0 4	0 3 0 1–0 7
Leucine	2 0 0 9–4 3	1 6 0 7–2 3	2 2 0 9–4 2
Isoleucine	1 1 0 4–2 4	1 0 0 4–1 1	1 2 0 4–2 4
Tyrosine	1 5 0 4–3 4	0 9 T–1 6	1 7 0 5–3 2
Lysine	2 7 1 7–3 7	3 2 1 2–5 2	2 8 1 4–5 4
Histidine	1 3 1 2–1 3	1 6 1 4–1 8	1 8 0 7–2 8
Phenyl alanine	2 0 0 5–3 4	0 9 T–1 6	1 1 0 2–2 3
Valine	2 9 1 4–5 8	2 5 1 4–3 5	4 0 1 6–8 6

T = Present in trace amount

bite his fingers lessened but the habit persisted when his splints and gloves were completely removed After a few minutes his fingers found their way to his mouth but were withdrawn immediately by the attendant On one occasion, he did bite his fingers

The patient received no special nursing care physical therapy etc His mother visited him frequently, but provided no special care or instruction She was shown how to accurately determine his daily intake by the use of a dietary balance The patient left the hospital weighing 11 2 kg that is, he gained 1 3 kg in the last month of his hospitalization His blood uric acid was 4 3 mg/100 ml and cerebrospinal fluid uric acid was 0 3 mg/100 ml

FOLLOW UP

The patient is attending our clinic every two weeks His mother brings a daily dietary sheet, which reports the daily intake of protein and calories Blood and urinary uric acid are measured at each visit plasma amino acids are determined less frequently

The patient s dietary intake has remained essentially as at the time of discharge, i e protein 38–70 g/day calories 1000–1500/day but his weight has not increased at the same rate At the time of writing the patient is 5 ¹/ years old he weighs 13 9 kg his height is 96 cm and his head circumference is 45 cm

The child can roll over He sits without support and can pull himself to a standing posi

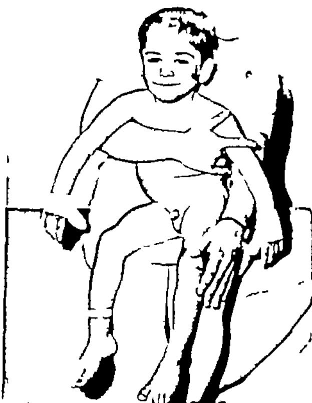


Fig 2 Patient after 6 weeks of treatment Note contrast to Fig 1

tion He also walks with support His gait is somewhat spastic but sensoring is virtually absent

His vocabulary has increased remarkably and he can phrase small sentences His disposition remains sunny He uses his hands but wears a splint on one arm and occasionally asks for the splint to be put on the other arm The splints are made of soft material which covers the forearm only and do not physically prevent the patient from bending his arms If the splints are removed while he is not preoccupied he objects He also asks for the splints whenever he becomes unhappy and has a temper tantrum

In order to evaluate effect of the allopurinol in the patient's progress allopurinol therapy has been periodically interrupted during the last four months The patient's mother receives a supply of capsules every two weeks allopurinol alternates with lactose Since the capsules of allopurinol are indistinguishable in every respect from those of lactose she does not know which one her son is getting Close questioning of the mother and examination of the child every two weeks have not indicated any significant change in the behaviour of the patient while off allopurinol therapy However the blood uric acid level drastically reduces when the patient is on allopurinol

DISCUSSION

Purine biosynthesis (Fig 3) starts with formation of inosinic acid monophosphate (IMP) from 2 phosphoribosyl 1 pyrophosphate (PRPP) and glutamine Inosinic acid in small amounts can be converted to adenylic acid (AMP) and guanylic acid (GMP) The two can later be reconverted to inosinic acid

The presence of adenylic acid and guanylic acid together act as a governor by feedback inhibition of the first step of the biosynthetic purine pathway (6) The efficiency of feedback inhibition can be greatly hampered by the absence of guanylic acid even in the face of a compensatory increase of adenylic acid

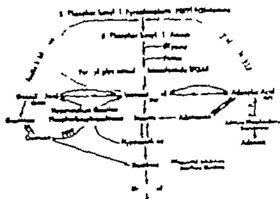


Fig 3 De Novo Purine Synthesis

Inosinic acid by dephosphorylation produces inosine which then yields the purine base hypoxanthine Hypoxanthine is partly salvaged from the system and reintroduced by the enzyme HG PRTase This salvage mechanism as well as guanylic and adenylic acids feedback inhibition probably plays a major role in the economy of the related nucleic acids Absence of the enzyme responsible for salvage of hypoxanthine and guanine allows the system to flood through the pathway to xanthine and eventually to uric acid In Lesch Nyhan Disease the enzyme HG PRTase required for the salvage mechanism is absent (13) Guanine cannot be converted to guanylic acid Perhaps because of the disrupted economy of inosinic acid and the possible decreased concentration of that substance guanylic acid is not formed This in turn releases the hand brake on the first step of de novo purine synthesis Thus marked acceleration in the rate of de novo purine biosynthesis results In Lesch Nyhan Disease the concentration of the partner of guanylic acid required for feedback inhibition i e adenylic acid is greatly increased as a compensatory mechanism

The elegant studies of Lesch & Nyhan in 1964 (5) showed that uniformly labeled glycine-C^{14} is incorporated into the urinary uric acid of these patients in amounts exceeding 200 times those of control subjects Patients with adult gout incorporated labeled glycine

C^{14} into uric acid only two or three times as much as normal

The salient biochemical features of Lesch-Nyhan Disease therefore consist of the following 1) Increased concentration of blood uric acid, resulting in excessive urinary output of that substance 2) Increased concentration of oxypurines intermediary metabolites of purine de novo synthesis, in CSF[7] and perhaps in brain 3) *Increased concentration of adenylic* acid in the red blood cells (13) and 4) Absence of the enzyme HG PRTase, *which can easily* be demonstrated by studies on the red cells *(13)*

The blood uric acid level of our patient ranged from 8 8 to 15 mg/100 ml before allopurinol therapy However the single determination performed at one year of age showed a value of 5 2 mg/100 ml which is around the upper limit of the conventionally accepted normal range The lesson to be learned in this respect is that blood uric acid although the most convenient test should not be used as the sole criterion for the diagnosis

We have not measured the oxypurines in the biological fluids of our patient but enzyme studies on the blood of our patient carried out through the courtesy of Dr Nyhan upheld our diagnosis The rate of production of IMP from hypoxanthine and GMP from guanine were respectively 0 03 % and 0 04 of normal which are indistinguishable from zero With the absence of HG PRTase established the possibility of loss of enzyme during shipment was examined through measurement of the sister enzyme A PRTase *The activity proved* to be 177 % of normal This compensatory increase of adenine enzyme is also a classic finding of the disease

The cardinal neurological manifestation relates to a malfunction in the area of the basal ganglia Rosenbloom *et al* (12) have recently shown that the basal ganglia of normal adults are particularly rich in HG PRTase They examined basal ganglia frontal lobe cerebellum spinal cord ovary liver spleen kidney muscle, pancreas *jejunum* and adrenal tissues from controls and found that basal ganglia had the highest activity Basal ganglia of one patient with Lesch Nyhan Disease tested by the same technique did not show any activity

The biochemical consequences of HG-PRTase deficiency are increased concentrations of uric acid, hypoxanthine xanthine, and probably adenine, as well as depletion of guanine and possibly of purine precursors from the cells *It is not known which of these secondary biochemical anomalies is responsible for the neurological symptoms or through* which mode of action the symptoms are produced

The most conveniently recognized biochemical abnormality is hyperuricemia, which can be expediently controlled by uricosuric agents Allopurinol which inhibits xanthine oxidase reduces blood uric acid at the expense of increased concentrations of xanthine and hypoxanthine It is doubtful that hyperuricemia per se plays any role in the production of symptoms other than thirst *After all none of* the neurological manifestations of the disease *have been observed in adult gout*

Our most recent observations indicate that periodic withdrawal of allopurinol therapy with the concomitant hyperuricemia does not change the patient s disposition nor cause recurrence of scissoring spasticity opisthotonus etc

Allopurinol therapy obviously reduces the chances of uric acid nephropathy and/or arthropathy On the other hand *it may* enhance the incidence of xanthine nephrolithiasis (1) *In any case* because of the absence of HG PRTase allopurinol cannot affect the excessive rate of purine de novo synthesis and/or establish the feedback inhibition (3)

A progressive deficiency state may play a major role in the production of symptoms Of 23 cases so far reported, 16 were below the 3rd percentile for weight 4 were at *the 3rd* percentile and 3 were between the 3rd and 10th percentiles Phrases such as hypocaloric dwarfism dying of inanition and difficulty in swallowing making feeding a real

problem (9) indicate that most of the patients if not all of them were severely malnourished at the time of the detection of the disease Little attention has been paid to the significance of this deficiency state and its devastating consequences Indeed in nine instances attempts were made to reduce blood uric acid by administration of a low purine low protein diet Growing brain tissue which uses protein synthesis for most of its activity may find it difficult to satisfy the growth demands of its semi starved cells Exaggerated de novo purine synthesis demanding additional molecules of glutamine glycine and aspartic acid for the formation of each additional molecule of uric acid cannot be disputed (2) Considering that for the formation of one molecule glutamine in this disease amounts to 500–1000 mg per day Although glutamine is not an essential amino acid the resources for its formation are not unlimited

Recent work by Paleologos *et al* (10) shows that in rats the intracellular concentration of alanine and glutamine is markedly decreased during gluconeogenesis The combined effects of starvation gluconeogenesis and excessive use of glutamine for exaggerated de novo purine synthesis must deplete the amino acid pool of the cell This intracellular deficiency state either alone or in combination with the effects of accumulated oxypurines is in our opinion responsible for productions of the neurological manifestations of the disease

In support of a deficiency state and the necessity for administration of glutamine is the persistently low concentration of that substance in blood prior to administration of MSG (Table ?) Moreover the marked improvement in the patient's clinical condition after institution of this therapy indicates that the deficiency state plays a major role in the production of the neurological manifestation of the disease Further work including estimation of the intracellular concentrations of amino acids in untreated Lesch Nyhan Disease and in controls should substantiate our contention

SUMMARY

A typical case of Lesch Nyhan Disease which responded favorably to a high protein diet and administration of monosodium glutamate has been presented The de novo purine synthesis has been briefly described The semi starved condition of these patients drinking mostly juice and water has been emphasized The role of the deficiency state in the production of symptoms and its correction have been discussed

ACKNOWLEDGEMENT

We are indebted to Dr W Nyhan for enzymic assay of the blood of the patient Acknowledgement is also due to Dr J Seegmiller for permission to reproduce a modified scheme of de novo purine biosynthesis from his article (13) We owe our thanks to Dr J Carr for uric acid determination in routine laboratory work up

REFERENCES

1 Greene M L Fujimoto W Y & Seegmiller J E Urinary xanthine stones—a rare complication of allopurinol therapy *New Eng J Med 280* 476 1969
2 Gutman A B & Yu T Uric acid metabolism in normal man and in primary gout *New Eng J Med 273* 252 1965
3 Kelley W N Rosenbloom F M Miller J & Seegmiller J E An enzymic basis for variation in response to allopurinol *New Eng J Med 278* 287 1968
4 Kornberg A Lieberman I & Simms E S Enzymic synthesis of purine nucleotides *J Biol Chem 215* 417 1955
5 Lesch M & Nyhan W L A familial disorder of uric acid metabolism and central nervous system function *Amer J Med 36* 561 1964
6 Magasanik B Regulation of the biosynthesis and interconversion of purine nucleotides *Arthrit Rheum 8* 610 1965
7 Newton E B Chromatogenic tungstate and its use in the determination of the uric acid of blood *J Biol Chem 120* 315 1937
8 Nugent C A & Tyler F H The renal excretion of uric acid in patients with gout and in non gouty subjects *J Clin Invest 38* 1890 1959
9 Nyhan W L Summary of clinical features *Fed Proc 27* 1034 1968
10 Paleologos G Muntwyler E & Kesner L Alanine and glutamine levels in rat liver tissue A direct relationship to gluconeogenic state *Proc Soc Exp Biol Med 132* 210 1969

11 Piez K A & Morris L A A modified procedure for automatic analysis of amino acids *Anal Biochem 1* 187 1960
12 Rosenbloom F M Kelley W N Miller J Henderson J F & Seegmiller J E Inherited disorder of purine metabolism *JAMA 202* 175 1967
13 Seegmiller J E Rosenbloom F M & Kelley W N Enzymic defect associated with a sex linked human neurological disorder and excessive purine synthesis *Science 155* 1682 1967
14 Spackman D H Stein W H & Moore S Automatic recording apparatus for use in the chromatography of amino acids *Anal Chem 30* 1190 1958

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Key words Lesch Nyhan disease hyperuricemia, treatment with monosodium glutamate

Acta Pædiat Scand 59 241–248 1970

THE RENAL TRANSPORT OF AMINO ACIDS IN UNTREATED INFANTS WITH PHENYLKETONURIA

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Excess of a single amino acid may produce changes in the transport and metabolism of several other amino acids This phenomenon of amino acid imbalance (9) has been considered to be effective in phenylketonuria (PKU) too and has been postulated to play an important role in the pathogenesis of this disease (10 12 17 20)

The data available however do not suffice to prove this hypothesis Reports of the literature are mostly concerned with the concentrations of free amino acids in the blood of phenylketonurics (10 12 20) Only scarce data are available for the intestinal transport of amino acids (21) and for the incorporation of amino acids into cells in the presence of high phenylalanine concentrations (3 8 14 23) The renal transport of amino acids seems to be not influenced as measurements of urinary excretion in phenylketonurics demonstrated (1 2 12 19) There are however only a few quantitative data of tubular reabsorption of amino acids in the presence of high tubular loading with phenylalanine (12) and none in infancy Therefore clearance studies were performed in infants with untreated phenylketonuria with simultaneous measurements of glomerular filtration rate As will be shown the high levels of phenylalanine in the blood and tubular fluid have no significant influence on the tubular reabsorption of phenylalanine and 15 other free amino acids

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METHODS

The studies were performed in 5 fullterm infants between the ages of 4 weeks and 15 months (infants no 2–6) and in one premature infant at the age of 3 weeks (infant no 1) Their age sex birth weight weight and body surface area at time of examination and the values of inulin and phosphate clearance and urinary volume are listed in Table 1 When the diagnosis of PKU was established the phenylalanine levels in the serum ran between 28 1 and 51 9 mg/100 ml All infants were considered to suffer from classical PKU and were treated successfully with a low phenylalanine diet subsequently

The clearance studies were performed just after diagnosis and before starting the treatment The procedures of the clearance examination were the same as described elsewhere (6) The glomerular filtration rate was measured with inulin The amino acids were determined in serum and urine by ion exchange chromatography as previously described (6) 12 infants aged 16 days to 4 months served as a control group Their data also were reported on previously (6)

RESULTS

The results of the clearance studies in 6 infants with PKU are given in Tables 2 and 3 The data include the concentrations of free amino acids in the serum (P_{AA} expressed in mg/100 ml) the urinary excretion rate ($U_{AA}V$ in µg/

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The data available, however, do not suffice to prove this hypothesis. Reports of the literature are mostly concerned with the concentrations of free amino acids in the blood of phenylketonurics (10, 12, 20). Only scarce data are available for the intestinal transport of amino acid (21) and for the incorporation of amino acids into cells in the presence of high phenylalanine concentrations (3, 8, 14, 23). The renal transport of amino acids seems to be not influenced as measurements of urinary excretion in phenylketonurics demonstrated (1, 2, 12, 19). There are, however, only a few quantitative data of tubular reabsorption of amino acids in the presence of high tubular loading with phenylalanine (12) and none in infancy. Therefore clearance studies were performed in infants with untreated phenylketonuria with simultaneous measurements of glomerular filtration rate. As will be shown, the high levels of phenylalanine in the blood and tubular fluid have no significant influence on the tubular reabsorption of phenylalanine and 15 other free amino acids.

METHODS

The studies were performed in 5 fullterm infants between the ages of 4 weeks and 15 months (infants no. 2–6) and in one premature infant at the age of 3 weeks (infant no. 1). Their age, sex, birth weight, weight and body surface area at time of examination and the values of inulin and phosphate clearance and urinary volume are listed in Table 1. When the diagnosis of PKU was established the phenylalanine levels in the serum ran between 28.1 and 51.9 mg/100 ml. All infants were considered to suffer from classical PKU and were treated successfully with a low phenylalanine diet subsequently.

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Table 1 *Some biographical data of the 6 infants with phenylketonuria in whom the amino acid clearance studies were performed*

Infant no	Initials	Sex	Birth weight (kg)	Age[a]	Weight (kg)	Surface area (m^2)	GFR[b] C_{In} (ml/min/1 73 m^2)	Phosphate clearance[b] (ml/min/1 73 m^2)	Urinary volume[b] (ml/min/1 73 m^2)
1 Premature	L F	♂	2 2	3 weeks	2 4	0 17	56	20 4	10 5
2	I J	♀	3 5	4 weeks	4 0	0 24	46	9 2	8 3
3	G B	♂	2 7	4 months	8 0	0 35	99	12 5	7 8
4	M P	♂	2 7	7 months	8 2	0 38	102	17 5	13 5
5	U K	♀	4 0	10 months	12 2	0 50	91	22 0	6 8
6	G W	♂	3 4	15 months	11 3	0 48	124	6 6	10 0

[a] At day of clearance study body surface area calculated from the nomogram according to the formula of DuBois and DuBois

[b] The values of the inulin-clearance (C_I) phosphate-clearance and urinary volume were obtained while determining the amino acid clearance

min/1 73 m) the endogenous renal clearance rates (C_{AA} in ml/min/1 73 m) and the percentage tubular reabsorption of amino acids (%T_{AA}) The mean values of the 5 fullterm infants (infant no 2–6)—the premature infant no 1 was excluded since prematures are known to have renal hyperaminoaciduria—are compared with the mean values of 12 normal infants (aged 16 days to 4 months) which is depicted in Figs 1–4

The serum concentration of phenylalanine ran between 28 1 and 51 9 mg/100 ml, with a mean value of 35 9 mg/100 ml (Table 2) The concentrations of most other amino acids tended to be lower in phenylketonurics than in the normals (Fig 1) This was especially valid for proline alanine methionine ornithine and histidine which had significantly decreased mean values ($p<0\ 02$) The other amino acids did not show significant changes

The urinary excretion showed the typical high values for phenylalanine (Table 2 and Fig 2) All other amino acids however were excreted in approximately the same or slightly lower amounts than in the normals

The values of the endogenous clearance rates were normal for phenylalanine and all other amino acids measured (Table 3 and Fig 3) The mean values of the 5 infants with PKU even tended to be lower than those of the normals This however can easily be explained by the fact that the mean age of the phenylketonurics was somewhat higher than that of the control group

The percentage tubular reabsorption of phenylalanine and the other free amino acids are listed in Table 3 and depicted in Fig 4

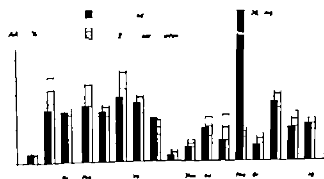


Fig 1 The concentrations of free amino acids in the serum of five infants with untreated PKU (mean values – black columns) in comparison with the values of normal infants (mean values and standard deviation – white columns) The decrease of proline alanine methionine ornithine and histidine are statistically significant ($p<0\ 02$)

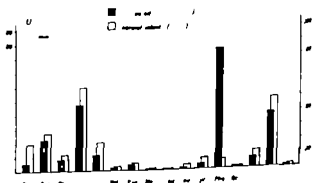


Fig 2 The urinary excretion of free amino acids in infants with untreated PKU (mean values – black columns) and in normal infants (mean values – white columns)

The mean values of percentage tubular reabsorption were within the normal range for almost all amino acids measured. The only exception seems to be phenylalanine which was reabsorbed even more completely in the phenylketonurics than in the normals.

DISCUSSION

To our knowledge direct measurements of renal clearances and tubular reabsorption of amino acids in PKU were reported only by Efron and coworkers (12). Their patients were 7 and 9 years old: one was untreated and the other one under low phenylalanine diet for 2 weeks. Other authors calculated the clearance rates of phenylalanine and other amino acids without simultaneous measurements of glomerular filtration rates (1, 19). Our results confirm the findings of these authors, i.e. the endogenous clearance rates of phenylalanine and other amino acids remain approximately the same despite the enormous elevation of blood phenylalanine levels in PKU.

Our results also confirm the observations that several amino acids in the blood are lower in PKU than in normals (10, 12, 17, 20). These findings have raised speculations concerning a possible pathogenetic role of amino acid imbalance in this disease. The data, however, are not uniform. In Table 4 those free amino acids are listed which were compared in the blood of phenylketonurics and normals by 4 groups of investigators. As can be seen a uniform statistically significant decrease ($p < 0.05$) was found only for alanine. The data of the other amino acids measured are variable.

It is our opinion that the decrease of amino acid in PKU is just a symptom of the disease and not a cardinal pathogenetic mechanism. It merely reflects different rates of amino acid uptake and metabolism by the tissues and possibly different rates of intestinal resorption (21) which may be the consequence of various enzyme stimulations by the high phenylalanine levels (22). It seems to be unspecific since

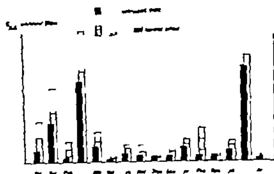


Fig. 3. The endogenous renal clearance rate of free amino acids in untreated PKU (mean values = black columns) and normal infants (mean values and standard deviation = white columns).

lowering of several amino acids in the blood can be provoked by many factors including painfull stimuli (30), saline or glucose infusion (5), imbalanced or deficient nutrition (13, 37), hormonal actions (18, 22, 25, 39), vitamin deficiency (16) and severe renal loss of amino acids (4). The reported effect of phenylalanine infusion also may be unspecific (12). An oral loading with leucine (24, 36), phenylalanine (10) and histidine (15) also produces comparable declines of several other amino acids in the blood.

For the renal physiologist there are some more aspects to be discussed. The tubular transport system for phenylalanine reabsorption seems to be very highly developed since a 30 to 50 fold overloading is handled as suffi-

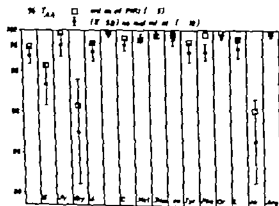


Fig. 4. The percentage tubular reabsorption of amino acids in infants with untreated PKU (mean values = white squares) and in normal infants (mean values and standard deviation = black dots with vertical lines).

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Infant no	Initials	Sex	Birth weight (kg)	Age	Weight[a] (kg)	Surface area (m^2)	GFR[b] C_{In} (ml/min/1 73 m^2)	Phosphate clearance[b] (ml/min/1 73 m^2)	Urinary volume[b]
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3	G B	♂	2 7	4 months	8 0	0 35	99	12 5	7 8
4	M P	♂	2 7	7 months	8 2	0 38	10[illegible]	17 5	13 5
5	U K	♀	4 0	10 months	12 2	0 50	91	22 0	6 8
6	G W	♂	3 4	15 months	11 3	0 48	1[illegible]4	6 6	10 0

[a] At day of clearance study body surface area calculated from the nomogram according to the formula of DuBois and DuBois
[b] The values of the inulin-clearance (C_{In}) phosphate-clearance and urinary volume were obtained while determining the amino acid clearance

min/1 73 m^2) the endogenous renal clearance rates (C_{AA} in ml/min/1 73 m^2) and the percentage tubular reabsorption of amino acids (% T_{AA}) The mean values of the 5 fullterm infants (infant no 2–6)—the premature infant no 1 was excluded since prematures are known to have renal hyperaminoaciduria—are compared with the mean values of 12 normal infants (aged 16 days to 4 months) which is depicted in Figs 1–4

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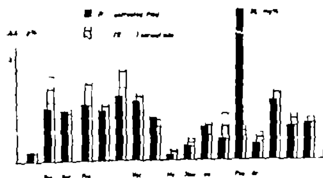


Fig 1 The concentrations of free amino acids in the serum of five infants with untreated PKU (mean values – black columns) in comparison with the values of normal infants (mean values and standard deviation – white columns) The decrease of proline alanine methionine ornithine and histidine are statistically significant ($p < 0.02$)



Fig 2 The urinary excretion of free amino acids in infants with untreated PKU (mean values – black columns) and in normal infants (mean values – white columns)

Table 3. *Values of endogenous clearance rates of free amino acids (C_{AA} in ml/min/1.73 m²) and of percentage tubular reabsorption (% T_{AA}) in 6 infants with untreated phenylketonuria*

The mean values of infant no. 2–6 are depicted in Figs. 3 and 4. The control [illegible] are derived from 12 infants aged 16 days to 4 months (6)

Endogenous clearance rates ml/min/1.73 m²

Amino acids	Infant no. 1	2	3	4	5	6	Controls $\bar{x}$	± S.D.
Thr	—	—	—	—	—	0.44	2.00	1.39
Ser	2.99	4.64	37	4.59	2.91	1.33	4.06	1.94
Pro	—	—	0.4	0.0	0.46	—	1.01	0.66
Gly	40.4	11.43	2.60	7.70	6.74	4.67	7.39	3.24
Ala	4.80	1.63	1.00	1.2	1.57	0.56	1.45	0.75
Val	0.44	0.27	0.34	0.10	0.24	0.13	0.23	0.1
Cys	2.23	0.87	—	0.54	0.39	—	1.07	0.27
Met	0.30	0.58	—	—	—	—	0.79	0.56
Ileu	—	0.18	0.16	0.73	0.28	0.55	0.90	0.16
Leu	—	0.33	—	0.24	0.33	0.58	0.79	0.53
Tyr	2.40	1.21	1.08	1.24	1.33	0.87	1.79	0.41
Phe	0.76	0.37	0.40	0.78	0.48	0.32	1.74	0.91
Orn	0.67	0.37	0.35	0.78	0.52	0.45	0.39	0.14
Lys	2.92	1.21	0.61	0.76	1.02	0.53	1.29	0.37
His	8.92	9.27	3.01	8.83	8.80	6.47	4.52	4.46
Arg	—	0.13	0.19	0.26	0.24	0.16	0.36	0.08

Percentage tubular reabsorption

Amino acids	Infant no. 1	2	3	4	5	6	Controls $\bar{x}$	± S.D.
Thr	—	—	—	—	—	99.63	96.97	1.08
Ser	94.64	99.91	97.61	95.50	96.80	98.93	93.41	2.65
Pro	—	—	99.76	100	99.50	—	98.25	1.39
Gly	61.66	75.16	97.37	92.94	[illegible]	96.23	87.57	4.44
Ala	91.43	96.44	98.99	98.76	[illegible]	99.45	97.34	1.10
Val	98.86	99.41	99.66	99.90	99.74	99.90	99.50	0.42
Cys	96.02	98.11	—	99.47	99.57	—	98.22	0.72
Met	99.46	98.91	—	—	—	—	98.79	0.90
Ileu	—	99.61	98.43	99.27	99.69	99.56	99.43	0.13
Leu	—	98.85	—	99.77	99.64	99.53	98.82	0.72
Tyr	95.71	97.57	98.91	98.79	98.54	99.90	97.23	1.14
Phe	98.64	99.0	99.60	99.24	99.47	99.74	97.36	1.01
Orn	98.80	99.40	99.65	99.24	99.43	99.64	99.37	0.27
Lys	94.79	97.37	99.38	99.26	98.88	99.57	97.80	1.14
His	84.07	79.85	94.91	91.44	90.33	94.78	96.62	5.07
Arg	—	99.78	99.81	99.75	99.74	99.87	99.41	0.21

Table 2 *Values of serum concentrations of free amino acids* (P_{AA} *in mg/100 ml) and of urinary excretion rates* ($U_{AA}V$ *in µg/min/1 73 m²) in 6 infants with untreated phenylketonuria*

The mean values of infant no 2–6 are depicted in Figs 1 and 2 The control values are derived from 12 infants aged 16 days to 4 months (6)

Amino acids	Serum concentrations mg/100 ml								Urinary excretion rate µg/min/1 73 m²							
	Infant no						Controls		Infant no						Controls	
	1	2	3	4	5	6	$\bar{x}$	±S D	1	2	3	4	5	6	$\bar{x}$	±S D
Thr	—	—	—	—	—	1 51	2 13	0 39	—	—	14 5	—	—	6 7	39 3	19 7
Ser	4 00	1 30	1 51	1 27	1 72	1 50	1 38	0 29	119 6	60 3	35 8	58 3	50 1	20 0	54 4	21 8
Pro	—	—	2 35	1 52	1 04	—	2 24	0 60	—	58 8	5 6	0 0	4 8	—	23 6	23 3
Gly	1 72	1 66	1 93	1 41	1 15	1 18	1 60	0 26	348 1	189 8	50 2	101 6	77 5	55 1	119 9	59 9
Ala	2 12	1 71	2 34	2 03	1 60	1 77	2 61	0 47	101 7	27 9	23 3	25 9	25 1	10 0	40 9	14 6
Val	1 66	1 72	1 86	2 23	1 52	1 30	1 89	0 45	10 7	4 7	6 3	2 2	3 6	1 7	5 6	2 8
Cys	1 20	1 38	—	1 18	1 24	—	1 00	0 21	26 8	12 1	7 7	6 4	4 8	—	10 6	3 7
Met	0 30	0 20	0 18	0 18	0 12	—	0 27	0 04	0 9	1 0	n d	n d	—	—	1 4	1 1
Ileu	0 46	0 32	0 54	0 57	0 43	0 31	0 52	0 11	—	0 6	0 9	4 3	1 2	1 7	1 3	1 0
Leu	0 82	0 70	1 97	0 91	0 72	0 57	1 02	0 28	—	3 7	—	2 2	2 4	3 3	7 1	4 0
Tyr	1 34	0 80	0 78	0 69	0 45	0 38	0 98	0 38	32 1	9 7	8 4	8 6	6 0	3 3	16 3	8 4
Phe	31 28	51 86	33 95	35 92	33 96	28 05	0 87	0 16	237 4	189 8	134 4	280 9	162 2	90 1	14 4	7 9
Orn	0 72	0 59	0 62	0 28	0 46	0 38	0 66	0 14	4 8	2 2	2 2	2 2	2 4	1 7	2 5	1 0
Lys	1 65	2 05	1 99	1 70	1 40	1 57	1 97	0 41	49 1	25 0	12 2	13 0	14 3	8 3	25 1	10 3
His	0 88	1 08	1 00	1 15	1 07	0 62	1 22	0 22	78 5	100 0	50 2	101 6	94 2	40 1	99 5	48 8
Arg	—	1 00	1 52	0 85	0 99	1 06	1 09	0 17	6 8	3 2	2 9	2 2	2 4	1 7	3 9	1 2

n d = not detectable

Table 2 *Values of serum concentrations of free amino acids* (P_{AA} *in mg/100 ml*) *and of urinary excretion rates* ($U_{AA}V$ *in μg/min/1.73 m²*) *in 6 infants with untreated phenylketonuria*

The mean values of infant no 2–6 are depicted in Figs 1 and 2 The control values are derived from 12 infants aged 16 days to 4 months (6)

Amino acids	Serum concentrations mg/100 ml								Urinary excretion rate μg/min/1.73 m²							
	Infant no						Controls		Infant no						Controls	
	1	2	3	4	5	6	P	±S D	1	2	3	4	5	6	P	±S D
Thr	—	—	—	—	—	1.51	2.13	0.39	—	—	14.5	—	—	6.7	39.3	19.7
Ser	4.00	1.30	1.51	1.27	1.72	1.50	1.38	0.29	119.6	60.3	35.8	58.3	50.1	20.0	54.4	21.8
Pro	—	—	2.35	1.52	1.04	—	2.24	0.60	—	58.8	5.6	0.0	4.8	—	23.6	23.3
Gly	1.72	1.66	1.93	1.41	1.15	1.18	1.60	0.26	148.1	189.8	50.2	101.6	77.5	55.1	119.9	59.9
Ala	2.12	1.71	2.34	2.03	1.60	1.77	2.61	0.47	101.7	27.9	23.3	25.9	25.1	10.0	40.9	14.6
Val	1.66	1.72	1.86	2.23	1.52	1.30	1.89	0.45	10.7	4.7	6.3	2.2	3.6	1.7	5.6	2.8
Cys	1.20	1.38	—	1.18	1.[illegible]4	—	1.00	0.21	26.8	12.1	7.7	6.4	4.8	—	10.6	3.7
Met	0.30	0.20	0.18	0.18	0.12	—	0.27	0.04	0.9	1.0	n d	n d	—	—	1.4	1.1
Ileu	0.46	0.32	0.54	0.57	0.43	0.31	0.52	0.11	—	0.6	0.9	4.3	1.2	1.7	1.3	1.0
Leu	0.82	0.70	1.97	0.91	0.72	0.57	1.02	0.28	—	3.7	—	2.2	2.4	3.3	7.1	4.0
Tyr	1.34	0.80	0.78	0.69	0.45	0.38	0.98	0.38	32.1	9.7	8.4	8.6	6.0	3.3	16.3	8.4
Phe	31.28	51.86	33.95	35.92	33.96	28.05	0.87	0.16	237.4	189.8	134.4	280.9	162.2	90.1	14.4	7.9
Orn	0.72	0.59	0.62	0.28	0.46	0.38	0.66	0.14	4.8	2.2	2.2	2.2	2.4	1.7	2.5	1.0
Lys	1.65	2.05	1.99	1.70	1.40	1.57	1.97	0.41	49.1	25.0	12.2	13.0	14.3	8.3	25.1	10.3
His	0.88	1.08	1.00	1.15	1.07	0.62	1.22	0.22	78.5	100.0	50.2	101.6	94.2	40.1	99.5	48.8
Arg	—	1.00	1.52	0.85	0.99	1.06	1.09	0.17	6.8	3.2	2.9	2.2	2.4	1.7	3.9	1.2

n d = not detectible

alanine—as could be suggested from the findings of van Stekelenburg (38)—or that some phenylalanine is desintegrated or bound during the passage through the tubular canals. Our present methods do not allow to investigate this further.

SUMMARY

In 5 fullterm infants aged 4 weeks to 15 months and in one premature infant aged 3 weeks with untreated phenylketonuria renal clearance studies were performed in order to determine the renal transport of phenylalanine and 15 other free amino acids. The data show that the high elevation of phenylalanine in the blood has no significant influence on the clearance rates and percentage tubular reabsorption of all amino acids measured. Phenylalanine seems to be reabsorbed even more completely in PKU than in normals. It is concluded that the tubular transport system for phenylalanine is characterized by a high specifity and high capacity, which is in the contrast to the tubular transport system for the imino acids and glycine.

In the serum the concentrations of some amino acids are lower in the presence of high phenylalanine levels. This finding, already reported by other authors before, is considered to be unspecific and without any significance for the pathogenesis of this disease.

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REFERENCES

1 Auerbach V. H., DiGeorge A. M. & Carpenter G. G.: Phenylalaninemia. In W. L. Nyhan (ed.): *Amino acid metabolism and genetic variation*. McGraw Hill Book Comp., New York, Toronto, London and Sydney 1967, p. 11.

2 Berry H. K., Umbarger B. & Livingston B.: Excretion of phenylalanine by normal children and by patients with phenylketonuria. *J Pediat 63*: 954, 1963.

3 Bickis I. J., Kennedy J. P. & Quastel J. H.: Phenylalanine inhibition of tyrosine metabolism in the liver. *Nature 179*: 11, 4, 1957.

4 Brass L., Brodehl J. & Gellissen K.: Renal glucose losing syndrome. *XII International Congress of Pediatrics*. Mexico 1968. Abstracts Vol III, p. 310.

5 Brodehl J.: *Der renale Transport der Aminosäuren im Säuglings- und Kindesalter*. Beiheft Arch. Kinderheilkunde No. 61. Ferd. Enke Verlag Stuttgart 1969.

6 Brodehl J. & Gellissen K.: Endogenous renal transport of free amino acids in infancy and childhood. *Pediatrics 42*: 395, 1968.

7 Brodehl J., Gellissen K. & Kowalewski S.: Isolierter Defekt der tubulären Cystin-Rückresorption in einer Familie mit idiopathischem Hypoparathyroidismus. *Klin Wschr 45*: 38, 1967.

8 Chirigos M. A., Greengard P. & Udenfriend D.: The uptake of tyrosine by rat brain in vivo. *J Biol Chem 235*: 2075, 1960.

9 Christensen H. N.: Metabolism of amino acids and proteins. *Ann Rev Biochem 22*: 233, 1953.

10 Colombo J. B. & Vassella F.: Das Aminosäurenspektrum im Plasma bei der Phenylketonurie. Beeinflussung durch L-Phenylalanin- und L-Thyrosinbelastung. *Klin Wschr 46*: 1215, 1968.

11 DeVries A., Kochwa S., Lazebnik J., Frank M. & Djaldetti M.: Glycinuria, a hereditary disorder associated with nephrolithiasis. *Amer J Med 23*: 408, 1957.

12 Efron M. L., Song Kang E., Visakorpi J. & Fellers F. X.: Effect of elevated plasma phenylalanine levels on other amino acids in phenylketonuric and normal subjects. *J Pediat 74*: 399, 1969.

13 Felig P., Owen O. E., Wahren J. & Cahill G. F. Jr.: Amino acid metabolism during prolonged starvation. *J Clin Invest 48*: 584, 1969.

14 Guroff G., King W. & Udenfriend S.: The uptake of tyrosine by rat brain in vitro. *J Biol Chem 236*: 1773, 1961.

15 Holton J. B.: The effect of a histidine load on plasma levels and renal clearances of other amino acids. *Clin Chim Acta 21*: 241, 1968.

16 Kaas W. P. & Brodehl J.: Nierenfunktion und renaler Aminosäurentransport beim Vitamin C Mangel (Moller-Barlowsche Erkrankung). 67. Tagung Deutsche Gesellschaft Kinderheilkunde Saarbrücken 1969. *Mschr Kinderheilk*. In press.

17 Knox W. E.: Phenylketonuria. In J. B. Stanbury, J. B. Wyngaarden & D. S. Fredrickson (eds.): *The metabolic basis of inherited disease*. Mc Graw Hill Comp., New York, Toronto, London and Sydney 1966, 2nd ed., p. 258.

18 Landau R. L. & Lugibihl K.: Effect of glucagon on concentrations of several free amino acids in plasma. *Metabolism 18*: 265, 1969.

19 Linneweh F. & Ehrlich M.: Die renalen und prärenalen Störungen des Aminosäuren-Stoffwechsels bei Phenylalanin-armer Ernährung. *Klin Wschr 38*: 904, 1960.

20 — Zur Pathogenese des Schwachsinns bei Phenylketonurie. *Klin Wschr 40*: 225, 1962.

Table 4 *Comparison of amino acid concentrations of blood in phenylketonurics and normal individ[uals] performed by 4 groups of investigation*

All statistically significant differences (with p values less than 0 05 [p < 0 05]) are listed
N = no difference ↓ = decrease ↑ = increase — = not determined

	Thr	Glu	Glu NH_2	Pro	Gly	Ala	Val	Cys	Met	Ileu	Leu	Tyr	Orn	Lys	His	Arg
Linneweh & Ehrlich 1962 PKU n = 14 normals n = 10	↓	—	—	—	—	—	↓	—	↓	↓	↓	↓	—	↓	↓	↓
Colombo & Vassella 1968 PKU n = 6 normals n = 8	—	↓	—	N	N*	↓	N	N	N	↓	N*	N*	—	N*	N*	↓
Efron et al 1969 PKU n = 20 normals n = 10	↓	N	↓	↓	N	↓	↓	↓	↓	↓	↓	—	—	—	—	—
Brodehl et al 1970 PKU n = 5 normals n = 12	—	—	—	↓	N	↓	N	N	↓	N	N	N	↓	N	↓	N

* Differences with p values in the range of 0 05 to < 0 1 which were considered as significant by Colombo & Vasse[lla] are rated here as N = no differences for reasons of comparison

ciently as the physiological load This is true not only for older children and adults but for infants also as our results demonstrate It seems quite impossible to reach a tubular maximum for phenylalanine reabsorption in man It was neither reached in dogs in an early study by Russo and coworkers (29)

The high tubular load with phenylalanine does neither produce any competitive nor inhibitory effect on the transport of other amino acids In analogy to the effects of lysine infusion (26) one would have expected that high phenylalanine loads would possibly compete with the transport of other neutral amino acids shared by the same transport system This however cannot be demonstrated

According to Scriver (34 35) and Rosenberg (28) and their coworkers there is much evidence that there exist at least two transport systems for reabsorption of a single amino acid in the renal tubules The one system is reported to have a high substrate specifity and a low capacity and the other one a low or group specifity but a high capacity for transport Both systems are controlled separately by different genes as studies of inborn errors—classical cystinuria (27) and isolated hypercystinuria (7) iminoglycinuria (31) and glycinuria (11)—revealed

Our findings suggest as Scriver (32) has [al]ready pointed out that the tubular reabsor[p]tion of phenylalanine is ruled by a transp[ort] system which is highly specific and also has [a] high capacity This is in contrast to the syste[m] described for the imino acids and glycine (3[ ] The transport system for phenylalanine d[e]monstrates an early ontogenic maturation [as] the results in the premature and young infa[nts] prove It is concluded that there are distin[ct] differences between the various tubular tran[s]port systems and that these have to be inves[ti]gated for each individual amino acid sep[a]rately

Finally it is astonishing that the percenta[ge] tubular reabsorption of phenylalanine (% T_r seems to be more complete in PKU than [in] normals In the normal infant % T_{Rx} is in [the] range of 97 36 ± 1 01 (6) In the fullte[rm] infants with PKU the values ranged betwe[en] 99 20 and 99 74 % and in the 3 weeks [old] premature infant with PKU the % T_{Rx} w[as] 98 64 % It is surprising that this more co[m]plete reabsorption is a constant finding in [the] 6 infants examined Since we have no kno[w]ledge of a systematic error in the analyti[cal] procedures it may be speculated that there [fur]ther exist some protein binding of phenyla[la]nine in high blood concentrations of phen[ylalanine]

alanine—as could be suggested from the findings of van Stekelenburg (38)—or that some phenylalanine is desintegrated or bound during the passage through the tubular canals. Our present methods do not allow to investigate this further.

SUMMARY

In 5 fullterm infants aged 4 weeks to 15 months and in one premature infant, aged 3 weeks with untreated phenylketonuria renal clearance studies were performed in order to determine the renal transport of phenylalanine and 15 other free amino acids. The data show that the high elevation of phenylalanine in the blood has no significant influence on the clearance rates and percentage tubular reabsorption of all amino acids measured. Phenylalanine seems to be reabsorbed even more completely in PKU than in normals. It is concluded that the tubular transport system for phenylalanine is characterized by a high specifity and high capacity which is in the contrast to the tubular transport system fo the imino acids and glycine.

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REFERENCES

1. Auerbach V H, DiGeorge A M & Carpenter G G. Phenylalaninemia. *In* W L Nyhan (ed.) *Amino acid metabolism and genetic variation*. McGraw Hill Book Comp. New York, Toronto, London and Sydney 1967, p. 11.
2. Berry H K, Umbarger B & Livingston B. Excretion of phenylalanine by normal children and by patients with phenylketonuria. *J Pediat* 63: 954, 1963.
3. Bickis I J, Kennedy J P & Quastel J H. Phenylalanine inhibition of tyrosine metabolism in the liver. *Nature* 179: 1124, 1957.
4. Brown L, Brodehl J & Gellissen K. Renal glucose losing syndrome. *XII International Congress of Pediatrics*, Mexico 1968. Abstracts Vol. III, p. 310.
5. Brodehl J. Der renale Transport der Aminosäuren im Säuglings- und Kindesalter. Beiheft Arch. Kinderheilkunde No. 61. Ferd. Enke Verlag, Stuttgart 1969.
6. Brodehl J & Gellissen K. Endogenous renal transport of free amino acids in infancy and childhood. *Pediatrics* 42: 395, 1968.
7. Brodehl J, Gellissen K & Kowalewski S. Isolierter Defekt der tubulären Cystin-Rückresorption in einer Familie mit idiopathischem Hypoparathyreoidismus. *Klin Wschr* 45: 38, 1967.
8. Chirigos M A, Greengard P & Udenfried D. The uptake of tyrosine by rat brain in vivo. *J Biol Chem* 235: 2075, 1960.
9. Christensen H N. Metabolism of amino acids and proteins. *Ann Rev Biochem* 22: 233, 1953.
10. Colombo J B & Vassella F. Das Aminosäurenspektrum im Plasma bei der Phenylketonurie-Beeinflussung durch L-Phenylalanin- und L-Tyrosinbelastung. *Klin Wschr* 46: 1215, 1968.
11. DeVries A, Kochwa S, Lazebnik J, Frank M & Djaldetti M. Glycinuria, a hereditary disorder associated with nephrolithiasis. *Amer J Med* 23: 408, 1957.
12. Efron M L, Song Kang E, Visacorpi J & Fellers F X. Effect of elevated plasma phenylalanine levels on other amino acids in phenylketonuric and normal subjects. *J Pediat* 74: 399, 1969.
13. Felig P, Owen O E, Wahren J & Cahill G F Jr. Amino acid metabolism during prolonged starvation. *J Clin Invest* 48: 584, 1969.
14. Guroff G, King W & Udenfried S. The uptake of tyrosine by rat brain in vitro. *J Biol Chem* 236: 1773, 1961.
15. Holtoe J B. The effect of a histidine load on plasma levels and renal clearances of other amino acids. *Clin Chim Acta* 21: 243, 1968.
16. Yaas W P & Brodehl J. Normalisierung und renaler Aminosäurentransport beim Vitamin C-Mangel (Moller-Barlowsche Erkrankung). 67. Tagung Deutsche Gesellschaft Kinderheilkunde, Saarbrucken 1969. *Mschr Kinderheilk*. In press.
17. Knox W E. Phenylketonuria. *In* J B Stanbury, J B Wyngaarden & D S Fredrickson (eds.) *The metabolic basis of inherited disease*. McGraw Hill Comp. New York, Toronto, London and Sydney 1966, 2nd ed., p. 258.
18. Landau R L & Lugibihl K. Effect of glucagon on concentration of several free amino acids in plasma. *Metabolism* 18: 65, 1969.
19. Linneweh F & Ehrlich M. Die renalen und prärenalen Störungen des Aminosäuren-Stoffwechsels bei Phenylalanin armer Ernährung. *Klin Wschr* 38: 904, 1960.
20. — Zur Pathogenese des Schwachsinns bei Phenylketonurie. *Klin Wschr* 40: 225, 1962.

21 Linneweh F Ehrlich M Graul E H & Hundeshagen H Über den Aminosauren Transport bei phenylketonurischer Oligophrenie *Klin Wschr 41* 253 1963
22 Meister A *Biochemistry of the amino acids* Academic Press New York and London 1965 2nd ed
23 Neame K D Phenylalanine as inhibitor of transport of amino acids in the brain *Nature 192* 173 1961
24 Nyhan W L Borden M & Childs B Idiopathic hyperglycinemia A new disorder of amino acid metabolism II The concentration of other amino acids in the plasma and their modifications by the administration of leucine *Pediatrics 27* 539 1961
25 Riggs T R & Walker L M Growth hormone stimulation of amino acid transport into rat tissues *in vivo J Biol Chem 235* 3603 1960
26 Robson E B & Rose G A The effect of intravenous lysine on the renal clearances of cystine arginine and ornithine in normal subjects in patients with cystinuria and Fanconi syndrome and their relatives *Clin Sci 16* 75 1957
27 Rosenberg L E Cystinuria genetic heterogeneity and allelism *Science 154* 1341 1966
28 Rosenberg L E Albrecht I & Segal S Lysine transport in human kidney evidence for two systems *Science 155* 1426 1967
29 Russo H F Wright L D Skeggs H R Tillson E K & Beyer K H Renal clearances of essential amino acids threonine and phenylalanine *Proc Soc Exper Biol & Med 65* 215 1947
30 Rouser G Jelinek B Samuels A J & Kinugasa K Free amino acids in the blood of man and animals I Method of study and the effects of venipuncture and food intake on blood free amino acids *in* J T Holden (ed) *Amino acid pools* Elsevier Publ Comp Amsterdam London and New York 1962 p 350
31 Scriver C R Renal tubular transport of proline hydroxyproline and glycine III Genetic basis for more than one mode of transport in human kidneys *J Clin Invest 47* 823 1968
32 — Use of human genetic variation to study membrane transport of amino acids in kidney *Amer J Dis Child 117* 4 1969
33 Scriver C R Efron M L & Schafer I L Renal tubular transport of proline hydroxyproline and glycine in health and in familial hyperprolinemia *J Clin Invest 43* 374 1964
34 Scriver C R & Muhyuddin F M Amino acid transport in kidney Heterogeneity of amino iso butyric uptake *J Biol Chem 243* 3707 1968
35 Scriver C R & Wilson O H Amino acid transport evidence for genetic control of two types in human kidney *Science 155* 1428 1967
36 Snyderman S E Cusworth D C Roitman E & Holt L E Jr Amino acid interrelationship The effect of variations in leucine intake *Fed Proc 18* 546 1949
37 Snyderman S E Holt L E Norton P M Roitman E & Phansalkar S V The plasma aminogram I Influence of the level of protein intake and a comparison of whole protein and amino acid diets *Pediat Res 2* 131 1968
38 Stekelenburg G J van & Desplanque J Deproteinization by ultrafiltration with centrifugal force *in Techniques in amino acid analysis* Technicon Instruments Comp Chertsey England 1966 p 51
39 Zimmermann Telschow H Bethge H Herberg L & Zimmermann H Untersuchungen am Menschen uber die Veranderungen der Aminosauren im Plasma im Verlauf des Insulin-Stress Testes *Klin Wschr 45* 768 1967

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PRIMARY HYPERPARATHYROIDISM IN CHILDREN

Brief Review of the Literature and a Case Report

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Primary hyperparathyroidism is a rare disease in children and only a few reports concerning its clinical manifestations have been published in the paediatric literature

In 1960 Nolan *et al* (31) reviewed briefly the 23 previously reported cases Wilkins (49) described the symptoms under four main headings 1) those due to hypercalcaemia 2) those resulting from the increased excretion of calcium and phosphorus in the urine 3) those related to changes in the skeleton and 4) metastatic calcification in soft tissues but he pointed out that under certain circumstances the typical symptoms and chemical changes of primary hyperparathyroidism may be altered

On the basis of the clinical picture in a 13 year old boy and an analysis of 42 paediatric patients with hyperparathyroidism reported in the literature we aim in this paper to elucidate the difficulties of differential diagnosis in this disease in children We would point out in particular that primary hyperparathyroidism should be considered in patients with diffuse symptoms such as general fatigue and weakness dizziness anorexia gastrointestinal bleeding cardiac arrhythmia or renal colic When kept in mind the diagnosis is usually not difficult to establish and primary hyperparathyroidism is fortunately one of those conditions which often can be cured by adequate surgical therapy However this diagnosis should be considered even when there is no large increase in the serum calcium level as will be shown below

CASE REPORT

P L a 13 year-old boy was reportedly in good health until one year prior to admission to hospital when he developed an abnormal increase in weight and daily diffuse headaches over the forehead Between the ages of 11 and 13 years he had grown only 3 cm in height but had almost doubled his weight The headaches which were relieved by salicylates were not preceded by any prodromal symptoms There were no abdominal pains no gastrointestinal or micturitional disturbances no increase in thirst and no polyuria the stools and urine seemed to be normal and there were no cardiac symptoms

Previously the boy had had measles rubella parotitis pertussis and chickenpox without complications He was the youngest of three brothers and was born in a normal delivery after a normal pregnancy birth weight 4070 g The development was normal and he had no difficulties at school His mother died of systemic lupus erythematosus when he was 12 years of age Otherwise the family was healthy and no tuberculous or allergic endocrine or nervous diseases were known among any of his relatives

The patient was admitted immediately as an inpatient for examination at the Paediatric Department of the University Hospital in Uppsala whence he was referred to the Department of Endocrinology of the

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Department of Medicine University Hospital Uppsala

Department of Endocrinology Karolinska Hospital Stockholm

Department of Paediatrics University Hospital Uppsala

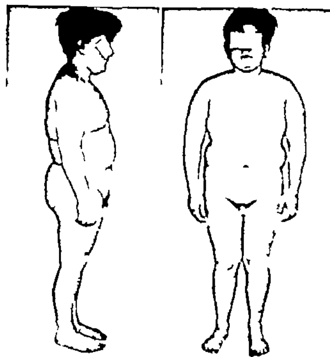


Fig 1 A 13 year old boy with obesity and primary hyperparathyroidism

Karolinska Hospital in Stockholm where hypophyseal and adrenal origins of the symptoms were excluded Because of slight hypercalcemia hyperparathyroidism was considered possible The examination was continued at the Department of Medicine of the University Hospital in Uppsala and operation with removal of a parathyroid adenoma was performed at the Department of Surgery of the latter hospital

Preoperative physical examination

When first examined the patient was 13 years old an apparently intelligent boy with a height of 139 cm (normal 140-165 for this age) and weight 56 kg (normal 25-40 kg for this height) There was pronounced truncal obesity (Fig 1) with an accumulation of fat between the scapulae and up to the back of the neck and striae over the hips No muscular atrophy was apparent but the finger joints allowed considerable hyperextension The blood pressure was 130/100 mm Hg The teeth gums mouth and throat thyroid gland heart lungs abdomen and neurological status were normal Incipient hair growth was noted over the pubic region and in the axillae The penis scrotum testes and prostate gland seemed to be rather small for his age

Laboratory findings

Haemoglobin 15 5 g/100 ml Erythrocytes 4 6 million/mm³ Leucocytes 6000/mm Erythrocyte sedimentation rate 5-12 mm/h Total serum protein (v Slyke) 7 5 g/100 ml Serum electrophoresis normal Total lipoids (Kunkel) 650 mg/100 ml Serum cholesterol 176-222 mg/100 ml Alkaline phosphatases 12 Bessey Lowry units (normal for this age up to 13 B L units) NPN 26 mg/100 ml

Glucose tolerance curve normal

PBI 8 μg/100 ml Triiodothyronine uptake in erythrocytes 14 % BMR −7−+27

Urinary excretion of 5 HIAA 9 mg/24 h GOT 30 units GPT 58 units Total gonadotropins normal (>5<20 MU/24 h)

Urinary excretion of 17 ketosteroids 3 0-7 0 mg/24 h 17 ketogenic steroids 5 4 23 mg/24 h Normal response to the metopirone and dexamethasone tests

Urine concentration test showed a maximal concentration capacity of 1 029 and an osmolarity of 1058 mosm i e normal values Sodium 139 148 mEq/l serum Potassium 3 4-4 3 mEq/l serum Chloride 103-108 mEq/l serum Calcium 10 8 11 4 mg/100 ml serum (5 4-5 7 mEq/l serum) Serum phosphorus 4 4 3 2 mg/100 ml

Intravenous infusion of 15 mg calcium per kg body weight during four hours did not change the level of the serum phosphorus values (4 5-4 7 mg/100 ml)

Urinary calcium 80-170 mg/24 h

Normal cerebrospinal fluid

Other examinations

Roentgenological examination of the skull showed normal conditions with a sella turcica of normal size and shape Cerebral pneumograms and X rays of the heart and lungs were normal Roentgenography of the skeleton showed a normal bone age of 13 years and open epiphyseal lines no osteoporosis On retroperitoneal air insufflation followed by abdominal aortography enlargement of the left adrenal gland could not be excluded In view of the possibility of a tumour this gland was explored and removed however it was found to be of normal size (weight 7 4 g) and histological examination showed normal adrenal gland tissue

A Mantoux test gave negative results EEG pathological with episodic abnormalities and no distinct foci ECG normal with no shortening of the QT distance Biopsy of the testes showed an adult picture with fully developed spermatogenesis

Further course and treatment

Because of a successive increase in the serum calcium values (to a maximum of 11 4 mg/100 ml serum or 5 7 mEq l) the parathyroid glands were explored and a parathyroid adenoma the size of a small gooseberry was found and removed Histological examination of the adenoma (Fig 2) showed parathyroid tissue with interspersed fat lobules and mainly built up of chief cells in solid accumulations or alveolar structures

After the operation the headaches disappeared and the patient's general condition improved The serum calcium values decreased to 9 9-10 6 mg/100 ml and the phosphorus values were 2 8-3 5 mg/100 ml There were no postoperative complications and no



Fig. 2. Parathyroid adenoma composed mainly of chief cells.

signs of hypocalcaemia. Probably due to the natural occurrence of puberty the boy increased in height and his obesity became less pronounced. His achievement at school and his general ability appeared normal during two years of observation after the operation.

Summary and discussion

The case presented here offered considerable diagnostic difficulties. The onset with increasing obesity and diffuse headache in association with the death of the boy's mother would appear to indicate a psychogenic origin of the symptoms, and a psychogenic element in the picture cannot be excluded.

The presence of an adrenal tumour as a reason for the boy's shortness of stature and obesity could not be excluded, and exploration and removal of the left adrenal gland were therefore performed, but normal conditions were found. At first, the slightly elevated blood calcium values aroused a suspicion of sarcoidosis, which diagnosis was not supported, however, either by the rest of the laboratory findings, roentgenological examinations or liver biopsy.

Preoperatively there appeared to be no strong reasons to consider a diagnosis of hyperparathyroidism. Apart from the elevated blood calcium values and unchanged phosphorus levels during calcium infusion there were no laboratory data to support the diagnosis and no classical symptoms or pathological roentgenographic findings. Nevertheless, the hypercalcaemia and the unclear picture in other respects were considered sufficient to motivate exploration of the parathyroid glands, which resulted in the final diagnosis: after the removal of a parathyroid adenoma the general condition of the patient improved, his headaches disappeared and the blood calcium values returned to normal.

DISCUSSION

On studying the literature, we found reports of altogether 42 children with primary hyperparathyroidism, the first being from the year 1930 (see Table 1). Including our own case the series reviewed here consists of 43 children (18 girls and 25 boys) of ages 0–15 years. (A number of further patients were reported to have hyperparathyroidism (3, 10, 13, 25 and

Table 1 *Primary hyperparathyroidism in children a summary of case reports from the literature*

Publication Name	Year	No	Sex M	Sex F	Age in years	Symptoms and signs	Serum calcium mg/100 ml	Serum phosphorus mg/100 ml	Urinary calcium mg/24 hrs	Findings at X ray examination	Duration of symptoms (yrs)	Autopsy	Operation	Histology Hyperplasia	Histology Adenoma	Histology Unknown
Philips (34)	1948	1			4/12	Vomiting dehydration	—	—	—	Extensive osteoporosis	4/12	×		×		
Randall & Lauchlan (39)	1963	2			9/12	Anorexia emaciation	20	2 3	—	Gen osteoporosis	9/12	×		×		
Pratt *et al* (35)	1947	3			10/12	Weakness constipation vomiting	22 1	3	—	Undercalcification irregularity of cortex	10/12	×		×		
Fretheim & Gardborg (17)	1965	4	×		1	Constipation mental retardation anorexia	24	2 3	—	Gen osteoporosis	1		×	×		
Roget & Beaudoing (42)	1947	5	×		2½	Vomiting constipation genu recurv	15	3 4	—	Gen osteoporosis resorpt of lamina dura renal calculi	2		×	×		
Crawford *et al* (11)	1956	6			3	Limp cherubic face	14	2 1	—	Osteoporosis cyst	1		×		×	
Lee *et al* (26) / Harmon (19)	1955 / 1956	7	×		7	Vomiting blindness B P 180/130	20	2 5	—	Osteoporosis renal lithiasis absent lamina dura	1		×		×	
Hanicka *et al* (18)	1964	8	×		7	Depression weakness	12	2 8	—	Fracture cysts	1		×	×	×	
Pugh (36)	1946	9	×		8	Polydipsia headache vomiting	19	2 8	—	Osteoporosis rarefactions	8/12	×	×			
Jonxis (23)	1955	10	×		9	Polyuria constipation	19	—	600	No renal lithiasis	2		×		×	
Timmons *et al* (48)	1955	11	×		10	Fracture vomiting abdominal pain	17	2 7	—	Renal lithiasis duodenal ulcer	1		×		×	
Nolan *et al* (31)	1960	12		×	11	Alopecia flaking of nails	13	2 8	300	Normal roentgen	2		×		×	
Wood *et al* (50)	1958	13			12	Polyuria thirst vomiting	17 5	2 1	—	Subperiosteal erosions osteoporosis	1½		×		×	
Fentz (16)	1961	14			12	Polyuria hypertension hemiparesis	17	—	360	Normal right carotid angiography	1/12		×		×	
Senna *et al* (44)	1963	15		×	12	Vomiting muscle weakness	15	2 5	—	Subperiostal resorption osteoporosis	2½		×		×	
Churchill & Cope (9) / Albright *et al* (2)	1934 / 1934	16		×	13	Thirst enuresis fracture bone pain	12	4 7	—	Susp renal stone cysts decalcification	4		×		×	
Anspach & Clifton (3)	1939	17		×	13	Polyuria fractures weakness convulsions	16 6	2 2	—	Osteoporosis	4		×			×
Bickel (5)	1945	18	×		13	Renal stones infantilism bradycardia	15 5	2 8	600	Renal lithiasis osteoporosis	2		×		×	
Arlt & Asang (4)	1953	19			13	Polyuria ileus goiter constipation	—	—	—	No skeletal changes	—	×			×	
Wondonoff *et al* (6)	1956	20	×		13	Ano ex a [illegible]	13 9	3 0	—	Renal l h a [illegible]	3		×		×	

Renfrank & Edwards (40)	1961	1			13	Polyuria ... weakness parathyroid crisis	—4	3 4		Decalcification subperiosteal resorption						
Rajasuriya et al (38)	1964				13	Irritability tumor of the neck	10 6	0	160	Absent lamina dura osteoporosis	3					
Chaves Carballo & Hayles (8)	1966	3			13	Hematuria swelling of both knees	15	8	340	Calculus of left ureter osteoporosis	2					
Pemberton & Geddie (33)	1930	4			14	Polyuria constipation enuresis	18	5	1500	Osteoporosis	4				×	
Dyke et al (14)	1933	25			14	B P 160/100 fractures dwarfism	18	2 8	1100	Osteoporosis	2/12		×		×	
Fahrni (15)	1940	26			14	Swelling of cheek epiphora	15 7	0	—	Osteoporosis cysts	1		×			
McClure & Lam (9)	1945	77			14	Knock knees tiredness	15	2 6	high	Osteoporosis	7/12					
Shallow & Fry (46)	1948	8			14	Weakness limp fracture	15 4	1 8	—	Osteoporosis	?		×		×	
Rienhoff (41)	1950	29			14	—	—	—	—	—	1		×			
Adam & Ritchie (1)	1954	30			14	Polydipsia swelling right knee	18 5	2 6	600	Osteoporosis	2		×		×	
Myers (30)	1963	31			14	Hematuria	15 4	3 4	—	Calculus in ureter osteoporosis	3/12		×	×		
Braun (7)	1964	32			14	Polyuria vomiting diplopia	17	2 7	high	Normal roentgen						
Chaves Carballo & Hayles (8)	1966	33		×	14	Hematuria	15 4	3 4	—	Renal lithiasis	2		×		×	
Ivemark (22)	1966	34			14	Vomiting goiter hematuria	15	2 9	520	Renal lithiasis	5/12		×		×	
Dyke et al (14)	1933	35			15	Limp fractures	17 8	—	970	Osteoporosis	3		×		×	
Drummer (12)	1933	36			15	Polyuria vomiting emaciation	19 4	4 0	—	Osteoporosis cysts	3		×		×	
Raco (37)	195.	37			15	B P 210/150 paralysis right arm	—	—	—	—	8/12	×				
Shaw et al (45) / Hoc et al (21)	1961 / 1961	38	×		15	Genu valgum	17	8	640	Osteoporosis	?		×		×	
Kinnaird et al (24)	1962	39		×	15	Symptoms of renal calculi	14	2 8	normal	Renal lithiasis normal skeletal roentgen	3		×		×	
Lloyd et al (77)	1965	40	×		15	Apathy knock knees	19 2	2 7	high	Deficient lamina dura osteoporosis	3		×		×	
Chaves Carballo & Hayles (8)	1966	41			15	Asymptomatic hypertension	13	2 0	470	Normal roentgen	?		×			
Lomnitz et al (78)	1966	42	×		15	Bone deformities	12	1 1	—	Absent lamina dura osteoporosis	2		×		×	
Bjernulf et al		43			13	Headache obesities	11 4	3 2	175	Normal skeletal roentgen	2		×		×	
Summary		43	25	18	0 15	(See table)	39	36	18	(See Table 2)	1/12–4	5	38	7	35	1
							Total no examined									

Table 1 *Primary hyperparathyroidism in children a summary of case reports from the literature*

Publication			Sex		Age									Histology		
Name	Year	No	M	F	in years	Symptoms and signs	Serum calcium mg/100 ml	Serum phosphorus mg/100 ml	Urinary calcium mg/24 hrs	Findings at X ray examination	Duration of symptoms (yrs)	Autopsy	Operation	Hyperplasia	Adenoma	Unknown
Philips (34)	1948	1			4/12	Vomiting dehydration	—	—	—	Extensive osteoporosis	4/12	×		×		
Randall & Lauchlan (39)	1963	2			9/12	Anorexia emaciation	20	2 3	—	Gen osteoporosis	9/12	×		×		
Pratt *et al* (35)	1947	3	×		10/12	Weakness constipation vomiting	22 1	3	—	Undercalcification irregularity of cortex	10/12	×		×		
Fretheim & Gardborg (17)	1965	4	×		1	Constipation mental retardation anorexia	24	2 3	—	Gen osteoporosis	1		×	×		
Roget & Beaudoing (42)	1947	5	×		2½	Vomiting constipation genu recurv	15	3 4	—	Gen osteoporosis resorpt of lamina dura renal calculi	2		×	×		
Crawford *et al* (11)	1956	6	×		3	Limp cherubic face	14	2 1	—	Osteoporosis cyst	1		×		×	
Lee *et al* (26) Harmon (19)	1955 1956	7	×		7	Vomiting blindness B P 180/130	20	2 5	—	Osteoporosis renal lithiasis absent lamina dura	1		×		×	
Hanicka *et al* (18)	1964	8	×		7	Depression weakness	12	2 8	—	Fracture cysts	1		×	×	×	
Pugh (36)	1946	9			8	Polydipsia headache vomiting	19	2 8	—	Osteoporosis rarefactions	8/12	×	×			
Jonxis (23)	1955	10	×		9	Polyuria constipation	19	—	600	No renal lithiasis	2		×		×	
Trumori *et al* (48)	1955	11	×		10	Fracture vomiting abdominal pain	17	2 7	—	Renal lithiasis duodenal ulcer	1		×		×	
Nolan *et al* (31)	1960	12			11	Alopecia flaking of nails	13	2 8	300	Normal roentgen	2		×		×	
Wood *et al* (50)	1958	13	×		12	Polyuria thirst vomiting	17 5	2 1	—	Subperiosteal erosions osteoporosis	1½		×		×	
Fentz (16)	1961	14			12	Polyuria hypertension hemiparesis	17	—	360	Normal right carotid angiography	1/12		×		×	
Senna *et al* (44)	1963	15		×	12	Vomiting muscle weakness	15	2 5	—	Subperiostal resorption osteoporosis	2½		×		×	
Churchill & Cope (9) Albright *et al* (2)	1934 1934	16			13	Thirst enuresis fracture bone pain	12	4 7	—	Susp renal stone cysts decalcification	4		×		×	
Anspach & Clifton (3)	1939	17			13	Polyuria fractures weakness convulsions	16 6	2 2	—	Osteoporosis	4		×			×
Bickel (5)	1945	18			13	Renal stones infantilism bradycardia	15 5	2 8	600	Renal lithiasis osteoporosis	[illegible]		×		×	
Arlt & Asang (4)	1953	19			13	Polyuria ileus goiter constipation	—	—	—	No skeletal [illegible]	—	×			×	
Bogdonoff *et al* ( )	1956	20	×		13	[illegible]	13 9	3 0	—	Renal [illegible]	3		×		×	

and minimal serum phosphorus levels in the 38 children of the series for whom these values were reported (see Table 1) Twenty eight of these 38 children had serum calcium values of 15 mg/100 ml or higher Among the 6 patients who were reported *later than 1965 (see Table* 1) the maximal serum calcium value was 15 4 mg/100 ml

The calcium excretion in the urine had been measured in 17 patients and in 12 of them the urinary calcium per 24 hours exceeded 400 mg

Renal function tests (NPN serum creatinine and maximal specific gravity of urine) were reported for 23 children In 11 of these the findings indicated impaired function but in no case were there signs of severe renal damage

As can be seen in Tables 1 and 3 35 chil dren (18 boys and 17 girls) had one adenoma In case 29 there were two adenomas and in case 39 the parathyroid adenoma was located within the thymus gland Seven patients had hyperplasia and in one of them (case 3) chief cell hyperplasia was noted All 7 patients with hyperplasia were boys and 4 of them were less

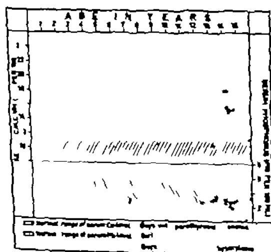


Fig 3 Maximal serum calcium and minimal serum phosphorus levels in 38 children with primary hyper parathyroidism (in 5 out of 43 cases no values of serum calcium or phosphorus were given, see Table 1)

Table 3 Classification of 43 children with primary hyperparathyroidism according to the findings at histological examination of the parathyroid glands and determinations of the serum calcium level

Parathyroid glands	Cases		Mean age (years)	Serum calcium >17 mg/100 ml	
	Total no	Boys ( )		No	
Adenoma	35	51	12 8	13 (out of 32)	41
Hyperplasia	7	100	3 2	5 (out of 6)	83
Unknown	1	0	13	0	0

than 2 years old (see Table 1) The serum cal cium values were much higher throughout in the patients with hyperplasia than in those with adenoma (see Table 3)

No case of parathyroid carcinoma was found in the literature reviewed

In adults primary hyperparathyroidism seems to be more common among women than men Norris (32) reviewed 336 cases from the literature and 60% of them were women Among the 138 patients with hyperparathy roidism reported by Hellstrom & Ivemark (20) there were 99 women (72) Similarly in another Swedish series (47) of 85 cases 69 were women Thus the ratio of women to men with hyperparathyroidism in adult series seems to be about 3 2 while the corresponding ratio in this paediatric series is 2 3 This find ing which demonstrates that hyperparathy roidism is more common among boys than among girls agrees with the observation of Hellstrom & Ivemark (20) that only 58 (19 out of 33) of the patients below 40 years of age were women

Hyperplasia of the parathyroids seems to be relatively more common in children than in adults This condition was found in 7 (19) of the 43 patients presented above while with hyperparathyroidism found only 8 5 with hyperplasia Hellstrom & Ivemark (20) re ported that 17 (12) of their 138 patients showed hyperplasia and Thoren & Werner

Table 2 *Incidence of main symptoms signs and X ray findings in the series of 43 children with primary hyperparathyroidism*

In 2 children (cases 29 and 37) there were no reports of X ray findings and in one of them (case 29) there was also no report of the symptoms and signs see Table 1

Symptoms and signs	No of cases according to age																	No of cases		
	Age in years																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	M	F	Total
Fatigue weakness anorexia constipation vomiting		3	1	1					3	1	1	1		2	4	5	2	15	9	24
Nervousness irritability depression headache		1							1	1					1		1	3	2	5
Mental retardation infantilism blindness dysarthria diplopia			1						1					1	2	1	1	6	1	7
Alopecia													1					0	1	1
Gastroduodenal ulcers												1				1?		2	0	2
Polyuria & polydipsia										1	1			2	4	3	1	6	6	12
Hypertension									1					1		2	2	4	2	6
Bone & joint symptoms fracture				1	1							1			3	6	4	6	10	16
X ray findings																				
Skeletal changes		3	1	1	1				2	1				2	6	7	5	17	12	29
Renal lithiasis				1					1			1			3 +1?	3	1	7	4	11
Renal lithiasis and skeletal changes				1					1						3	1		5	1	6

43) but the diagnosis in these cases is considered uncertain and they are therefore not included in our study)

A review of the 43 patients is presented in Table 1

Table 2 shows the incidence of the main symptoms and X ray findings in the 43 patients

The predominating symptoms were weakness anorexia and irritability which were present in more than 50 of the patients Constipation was noted in 7 children and polyuria and polydipsia in 12 Local skeletal changes were observed in 21 and renal calculi in 11 of the 43 patients

Complete heart block was noted in case 7 (Table 1) while in no other case was shortening of the Q T interval observed

Three patients had had parathyroid crises (Table 1 cases 7 14 and 21) their serum calcium levels lay at 20 17 and 22 mg/100 ml respectively Residual cerebral and renal lesions were noted in case 7 probably due to the long duration of the disease before operation In case 14 there was immediate and complete improvement after the operation both clinically and as regards the laboratory findings The postoperative improvement was also satisfactory in case 21

EEG recordings were made in 7 children The tracings were clearly pathological in case 14 with parathyroid crises which on two occasions led to coma and temporary hemiparesis seven months after the operation however a distinct improvement of the EEG pattern was noted This patient was described as a case of hypertensive encephalopathy and had a blood pressure of 180/110 mm Hg during the crises In the youngest patient with parathyroid crises (case 7) on the other hand the EEG tracings showed deterioration after operation with a diffusely irregular pattern and the boy became blind No EEG recordings were made in the oldest patient with parathyroid crises (case 21) In one child (case 40) the EEG pattern indicated a non specific dysfunction in the upper part of the brain stem In our patient (case 43) there were minor abnormalities such as varying episodes with no distinct foci and polymorphic episodes over the temporal lobes In three patients (cases 3 4 and 32) the EEG tracings showed nothing abnormal

Fig 3 shows the maximal serum calcium

and minimal serum phosphorus levels in the 38 children of the series for whom these values were reported (see Table 1) Twenty eight of these 38 children had serum calcium values of 15 mg/100 ml or higher Among the 6 patients who were reported later than 1965 (see Table 1) the maximal serum calcium value was 15 4 mg/100 ml

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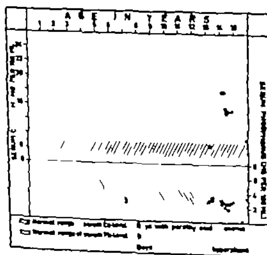


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Hyperplasia of the parathyroids seems to be relatively more common in children than in adults This condition was found in 7 (19) of the 43 patients presented above while with hyperparathyroidism found only 8 5% with hyperplasia Hellstrom & Ivemark (20) reported that 17 (12%) of their 138 patients showed hyperplasia and Thoren & Werner

(47) gave a figure of 6 (7%) out of 85 All 7 patients with cellular hyperplasia in the present series of 43 children were boys, and mainly from the lowest age groups

The renal lesions seem to be less pronounced in children than in adults and the incidence of renal calculi lower reasonably due to a shorter duration of the disease in children Only 11 (25%) of the 43 paediatric patients had renal calculi which may be compared with 86% of the adult series of Hellstrom & Ivemark (20) and 59% of the adult series of Thoren & Werner (47)

Primary hyperparathyroidism has been said to give a higher incidence of skeletal changes and a higher degree of hypercalcaemia in children than in adults Thus local skeletal changes were reported in 20 of the 43 paediatric patients presented here while Thoren & Werner (47) observed such changes in only 9 out of 85 adult patients

Thoren & Werner (47) conclude that the increase in the number of diagnosed adult cases of primary hyperparathyroidism noted in recent years mainly concerns patients with renal calculi and atypical symptoms (such as fatigue weakness muscular adynamia and constipation) while the number of cases with skeletal involvement seems to remain essentially unchanged

The clinical picture of primary hyperparathyroidism is often non-specific and inconspicuous The diagnosis thus has to be based upon laboratory findings mainly the serum calcium level This level sometimes can be only moderately elevated as is illustrated by the cases presented above It may perhaps be significant that the maximal serum calcium level was not higher than 15 4 mg/100 ml in any of the 6 cases diagnosed later than in 1965

In order to avoid severe and sometimes irreparable damage as a result of hyperparathyroidism an early diagnosis seems to be essential and the serum calcium level ought to be measured in children with unexplained symptoms such as anorexia, weakness, constipation and increased thirst

SUMMARY

A case of primary hyperparathyroidism in a 13 year old boy is reported showing some difficulties in diagnosing this disorder and pointing out that the disease may occur with only minor increases in the serum calcium values

Forty two children with hyperparathyroidism have been found in the literature, and some data for these patients are also presented and discussed

In children primary hyperparathyroidism is more common among males than females (ratio 3 2) while in adults this sex relationship is reversed (ratio 2 3) In about one fifth of the children (all boys) the condition was due to diffuse enlargement of the parathyroids these boys also showed higher serum calcium levels than the patients with adenoma In adults diffuse hyperplasia occurs in only one-tenth of the patients

Primary hyperparathyroidism seems to be accompanied by a higher incidence of skeletal changes and, as a rule a higher degree of hypercalcaemia in children than in adults, while renal lesions seem to be less pronounced and the incidence of renal calculi lower

General symptoms of weakness such as anorexia muscular adynamia and constipation as well as increased thirst in children should arouse suspicion of hyperparathyroidism and the serum calcium level should be determined so that an early diagnosis may be made and treatment given

REFERENCES

1 Adam A & Ritchie D Hyperparathyroidism with increased bone density in the areas of growth *J Bone Joint Surg (Amer) 36 B* 257 1954

2 Albright F Aub J C & Bauer W Hyperparathyroidism a common and polymorphic condition as illustrated by seventeen proved cases from one clinic *JAMA 102* 9 1934

3 Anspach W E & Clifton W M Hyperparathyroidism in children report of two cases *Amer J Dis Child 58* 540 1939

4 Arlt H G & Asang E Intestinaler Hyperparathyroidismus *Z Kinderheilk 73* 294 1953

5 Bickel G Démonstrations cliniques *Helv Med Acta 12* 273 1945

6 Bogdonoff M D Woods A A White J E & Engel F L Hyperparathyroidism *Amer J Med 1* 538 1956

7 Brunn I Primaer hyperparathyroidisme med neurologiske symptomer Et tilfaelde hos en 14-årig dreng *Ugeskr Laeg 127/1* 13 1964

8 Chaves-Carballo E & Hayles A B Parathyroid adenoma in children Report of three cases with unusual articular manifestations in one case *Amer J Dis Child 112* 553 1966

9 Churchill E D & Cope O Parathyroid tumors associated with hyperparathyroidism *Surg Gynec Obstet 58* 255 1934

10 Cooley T B Hyperparathyroidism and similar diseases of bone *Trans Amer Pediat Soc 43* 70 1931

11 Crawford D J M Stefanelli J & Alvarez, A F Three unusual cases of hyperparathyroidism *Brit J Surg 44* 193 1956

12 Dresser R Osteitis fibrosa cystica associated with parathyroid overactivity *Amer J Roentgen 30* 596 1933

13 Dirken J Beitrag zur Kenntnis der malacischen Erkrankungen des kindlichen Skelettsystems *Z Kinderheilk 46* 114 1928

14 Dyke S C Walker R M & Freeman E Adenoma of the parathyroid associated with generalized osteitis fibrosa *Lancet II* 530 1933

15 Fahrni G S Parathyroid tumors report of 2 new cases and discussion of these and 5 cases previously reported *Trans Amer Goiter Ass* 252 1940

16 Fentz V Hypertensive encephalopathy in a child *Acta Neurol Scand 37* 307 1961

17 Fretheim B & Gardborg O Primary hyperparathyroidism in an infant *Acta Chir Scand 129* 557 1965

18 Hanecka M Jelonek A Moskala D & Badowski Z Primary hyperparathyroidism in a 7-year-old boy *Endokr Pol 15* 27 1964

19 Harrison M Parathyroid adenoma in a child *Amer J Dis Child 91* 313 1956

20 Hellström J & Ivemark B I Primary hyperparathyroidism clinical and structural findings in 138 cases *Acta Chir Scand Suppl* 294 1962

21 Hioco D Bordier Ph Mazabraud A De Sèze S & Lichtwitz, A Cinq observations d'osteose parathyroidienne adenomateuse Confrontations cliniques radiologiques anatomiques et biochimiques *Presse Med 69/1* 153 1961

Ivemark B I Personal communication 1966

23 Jonxis J H P Een Geval van Hyperparathyroidie op de Kinderleeftijd *Nederl T Geneesk 99 I* 690 1955

24 Kinnard D W Maurer J E & Bryan J E Hyperparathyroidism due to an intrathyroidal parathyroid adenoma *Southern Med J (Bghum Ala)* 55 870 1962

25 Landon J F Parathyroidectomy in generalized osteitis fibrosa cystica. *J Pediat* 1 544 1932

26 Lee C M McElhinney W T & Goll E A Unusual manifestations of parathyroid adenoma *Arch Surg (Chicago) 71* 475 1955

27 Lloyd H M Aitken R E & Ferrier T M Primary hyperparathyroidism resembling rickets of late onset *Brit Med J II* 853 1965

28 Lomnitz, E Sepulveda L & Stevenson C Primary hyperparathyroidism simulating rickets *J Clin Endocr 26* 309 1966

29 McClure R D & Lam C R End results in the treatment of hyperparathyroidism *Ann Surg 121* 454 1945

30 Myers N A Parathyroid adenoma *Med J Aust 50/1* 735 1963

31 Nolan R B Hayles A B & Woolner L B Adenoma of the parathyroid gland in children Report of case and brief review of the literature *Amer J Dis Child 99* 622 1960

32 Norris E H The parathyroid adenoma A study of 322 cases *Surg Gynec Obstet 84* 1 1947

33 Pemberton J de J & Geddie K B Hyperparathyroidism *Ann Surg 9* 207 1930

34 Philips R N Primary diffuse parathyroid hyperplasia in an infant of four months *Pediatrics* 429 1948

35 Pratt E L Geren B B & Neuhauser E B D Hypercalcemia and idiopathic hyperplasia of the parathyroid glands in an infant. *J Pediat 30* 389 1947

36 Pugh R J Primary hyperplasia of the parathyroids in a boy aged 8 years *Proc Roy Soc Med 39* 694 1945-1946

37 Race G J Primary hyperparathyroidism masked as primary renal disease with hypertension *N C Med J 13* 659 1952

38 Rapoport A Perin O A Ratnaike V T & de Fonseka C P Parathyroid adenomata in childhood *Am J Dis Child 107* 442 1964

39 Randall C & Lauchlan S C Parathyroid hyperplasia in an infant *Am J Dis Child 105* 364 1963

40 Reinfrank R F & Edwards T L Parathyroid crisis in a child *J Am Med Ass 178 II* 468 1961

41 Rienhoff W F Jr The surgical treatment of hyperparathyroidism with a report of 27 cases *Ann Surg 131* 917 1950

42 Roger J & Beaudoing A Un cas d'hyperparathyroidie primitive chez un enfant de 30 mois *Pediatrie 14* 21 1947

43 Sano T Hirooka M Hayashi Y Koseki E & Tanabe S Primary hyperparathyroidism in childhood a case report *Ann Paediat Fenn 4* 14 1958

44 Scmas M S Bilbo R E & Smith D W Parathyroid adenoma in a child *J Pediat 62* 876 1963

45 Sèze S de Lichtwitz, A Hioco D Bordier Ph & Parlier R Tests biochimiques d'exploration de la fonction parathyroidienne dans les hyperparathyroidies primitives *Rev Rhum 28* 483 1961

46 Shallow T A & Fry K E Parathyroid ade

noma occurence in father and daughter *Surgery 24* 1020 1948
47 Thoren L & Werner I Hyperparathyroidism clinical observations in a series of 85 patients *Acta Chir Scand 135* 395 1969
48 *Tsumori H Jensen E Hunnicutt A J Fore*man N & Kinsell L W Juvenile hyperparathyroidism in association with peptic ulcer *J Clin Endocr 15* 1141 1955
49 Wilkins L *The diagnosis and treatment of endocrine disorders in childhood and adolescence* Blackwell Scientific Publications Oxford 1957 2nd edition
50 Wood B S B George W H & Robinson A W Parathyroid adenoma in a child presenting as rickets *Arch Dis Child 33* 46 1958
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Acta Paediat Scand 59 259–264 1970

THE INFLUENCE OF ADENINE ON THE CLINICAL FEATURES AND PURINE METABOLISM IN THE LESCH NYHAN SYNDROME

S P M VAN DER ZEE E J P LOMMEN J M F TRIJBELS and E D A M SCHRETLEN

From the Paediatric Department University of Nijmegen Nijmegen the Netherlands

The Lesch Nyhan syndrome is characterized by mental retardation choreoathetosis aggressive behaviour with automutilation and hyperuricaemia (9 17 18) A number of authors have mentioned the occurrence of a megaloblastic anaemia in this syndrome (7 11 12 18 24) The syndrome is an X linked recessive disorder (13 16 23) The purine synthesis de novo is greatly increased in these patients (9 15) Seegmiller found a deficiency of the enzyme hypoxanthine guanine phosphoribosyl transferase in patients with this syndrome (22) The relationship between the greatly increased purine synthesis de novo and this enzyme deficiency is at present an important feature of investigation (20)

We studied the influence of orally administered adenine on the clinical features (25) and on the purine metabolism of two patients with the Lesch Nyhan syndrome

METHODS

During the time of the investigation two patients with this syndrome Re W and Ru W (Fig 1 no 3 and 5) were given a purine free diet The uric acid was determined according to the enzymatic spectrophotometric method (10) The glycine 2 ^{14}C was orally administered in a dose of 5 μC to Re W and 4 μC to Ru W For the first two days after administration of glycine ^{14}C urine was collected in 12 hour periods, thereafter in 24-hour periods The uric acid was isolated from these samples purified and the ^{14}C activity of uric acid was measured according to the method of Lesch & Nyhan (9) The activity of the enzyme hypoxanthine guanine phosphoribosyl transferase was measured according to the method of Carter (2)

RESULTS

We have already described the symptoms of the boys in family W H W (no 1) Re W (no 3) and Ru W (no 5) (Fig 1) (24) The most important clinical data and laboratory results obtained from Re W and Ru W are shown in Table 1 The results of the investigation of the purine metabolism in these patients are shown in Tables 2 and 3 We found no activity of the enzyme hypoxanthine guanine phosphoribosyltransferase in the erythrocytes of Re W and Ru W (Table 4) The haematological findings are shown in Table 5

Bone marrow Re W

The bone marrow showed a normal ratio of erythropoiesis and leucopoiesis As well as a normoblastic there was also a megaloblastic maturing sequence In the megaloblasts a regularly divided chromatine structure was visible The erythropoiesis showed an inhibition of nuclear division (Fig 3) In the white sequence macromyelocytes were also present

Bone marrow Ru W

The bone marrow of this youngest patient showed a normal ratio of erythropoiesis and leucopoiesis The red sequence seemed to be normal There were no convincing megalob-

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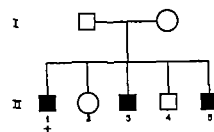


Fig 1 Family of our patients

lasts however, the young cells showed finely divided chromatine structure The leucopoiesis seemed normal the number of megakaryocytes was normal

None of the patients received anticonvulsive therapy in the form of diphantoin or mysoline

Table 1 *The most important clinical data and laboratory results in patients Re W and Ru W*

Date of birth	Re W 3 8 1959	Ru W 14 3 1966
Age at the time of the investigation	9 years	2 years
Mental retardation	+	+
Choreoathetosis	+	+
Automutilation	+	+
Serum urea mg/100 ml	48–59	41–44
Serum creatinine mg/l	6 1–7 1	4 4–4 7
Urine protein	Negative trace	Negative trace
sediment	Uric acid Crystals	Uric acid Crystals
cultures	Negative	Negative
Urea clearance ml/min/1 73 m²	21 9–24 9	28 0–41 0
Endogeneous creatinine clearance ml/min/1 73 m	45 0–59 7	45 2–59 2
Concentration ability	sw 1013 osmol 364	sw 1022 osmol 640
Phenolred excretion after 2 hours	65	60
Phosphate-clearance in of the creatinine-clearance	57–77	86–89
I V pyelogram	No anatomical aberration	No anatomical aberration
Glucosuria	Negative	Negative
Acid load with 100 mEq NH_4Cl per m² per 24 hours expressed in indices according to Elkington (15)		
Norm		
T A 3rd day 0 6–1	0 34	0 45
5th day 0 4–1	0 21	
NH_4 3rd day 0 8–2 4	0 74	0 90
5th day 0 9–2 7	0 80	
H 3rd day 1 4–3 4	1 0	1 36
5th day 1 5–3 4	0 85	

Table 2 *The serum uric acid concentrations and urinary uric acid excretions in patients Re W and Ru W*

	Re W	Ru W	Controls
Serum uric acid mg/100 ml	8 6–11 7	8 9–10 3	3 0–4 5
Uric acid excretion in urine mg/24 hours	642–714	456–600	176–789[a]
Uric acid excretion mg/kg/24 hours	43 4–48 2	42 2–55 5	10 3[a]
Excretion ratio uric acid/creatinine/24 hours	1 6–2 4	1 8–3 1	0 66[b]

[a] Published data from Nyhan (2)
[b] Published data from Kaufman e a (17)

Table 3 *The cumulative excretion of isotope in urinary uric acid after oral gift of glycine ¹⁴C in patients Re W and Ru W*

	Re W	Ru W	Controls
7 days recovery of isotope in urinary uric acid	2 48	1 63	0 1[a]

[a] Published data from Nyhan who administered the glycine ¹⁴C intraveneously

Table 4 *Activity of adenine and hypoxanthine guanine phosphoribosyltransferase in haemolysates of erythrocytes of controls and patients Re W and Ru W*

The controls had no metabolic neurological or haematological disorders

	Adenine and hypoxanthine guanine-phosphoribosyltransferase activity in mμmol/ml red cells/min		
	Substrate adenine	Substrate guanine	Substrate hypoxanthine
Controls (mean value ± 1 s d)	134±42	710±121	323±91
Patient Re W	180	Non detectable	Non detectable
Patient Ru W	241	Non detectable	Non detectable

The oldest boy in this family H W who died at two and half year age had also a megaloblastic anaemia Both patients H W and Re W showed no improvement in their megaloblastic anaemia after intramuscular injection

Table 5 *Haematological findings in patients Re W and Ru W*

	Re W	Ru W
Age at the time of investigation	9 years	2 years
Hb gr/100 ml	8 4–9 0	11 6–13 1
Erythrocytes mill mm³	1 64–1 83	3 36–3 53
Hematocrit %	25–27	36 5–39 5
Reticulocytes ‰	7 14	15–14
MCHC %	33 5–33	31 5–33
MCV µ	153–147	108–112
MCH γ µg	52–49	35–37
Leucocytes mm³	9 400–10 700	7 000–10 700
Thrombocytes mm	64 000–314 000	184 000–388 000
TIBC µgr/100 ml	436	435
Serum iron µgr/100 ml	61	107
Iron saturation	1	24
Osmotic resistance 50 haemolysis	0 48 NaCl	0 48 NaCl
Autohaemolysis	Normal	Normal
Survivaltime of own erythrocytes T½ Cr	31 days	
Histamine fast achlorhydria	Absent	Absent
Vitamine B_{12} (norm. 90–760 pg ml)	290	300
Folic acid mµ g ml (norm 7 30 mµg/mg)	6 5	9 6

of 25 µgr vitamine B_{12} per day and oral administration of 15 mg folic acid per day Oral administration of adenine to patient Re W resulted in a disappearance of the megaloblastic anaemia (Fig 2)

Three months after the adenine therapy was terminated the bone marrow once again showed a megaloblastosis (Fig 3)

We continued to study in this patient and in his brother the effect of the administration of adenine on purine synthesis de novo as measured by incorporation of glycine ¹⁴C into urinary uric acid Indeed we found a diminished incorporation during administration of adenine (Table 6 Figs 4 and 5)

DISCUSSION

These brothers show the already classical feature of the Lesch Nyhan syndrome (9 17 18)

The results of our investigation of the hyperuricaemia the hyperuricuria and the increased glycine ¹⁴C incorporation in urinary uric acid are in close correspondence with those of the other investigators (5 6 7 8 9 14 15 21 22)

The kidney function disorders which we diagnosed agree very well with the findings of Marie and Royer and according to them fit in with the picture of a chronic interstitial nephritis (12 21)

In H W and Re W we found a megaloblastic anaemia refractory to vitamine B_{12} or folic acid therapy In the 3rd patient of the

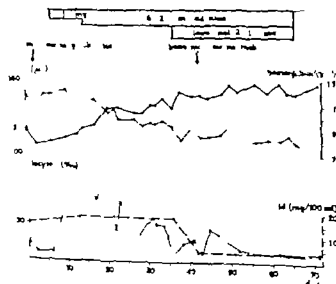


Fig 2 Response of megaloblastic anaemia to orally administered adenine in patient Re W



Fig 3 Bone marrow of Re W before *a* during *b* and 3 months after *c* termination of adenine administration

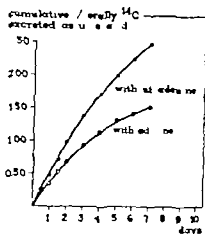


Fig 4 The cumulative excretion of isotope in urinary uric acid after oral gift of glycine C in patient Re W before and during adenine administration dose adenine 20 mg/kg body weight/day

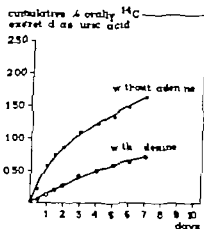


Fig 5 The cumulative excretion of isotope in urinary uric acid after oral gift of glycine C in patient Ru W before and during adenine administration dose adenine 50 mg/kg body weight/day

family Ru W the megaloblastosis was less pronounced

On the ground of our investigation and those of other authors (1 7 8 11 12 18) we believe that a megaloblastic anaemia is a characteristic symptom of the Lesch-Nyhan syndrome No indication of a vitamine B_1 deficiency was found in any of the patients with megaloblastic anaemia

Kelley found a decreased folic acid concentration in the serum of his patients (7) Owing to the fact however that in a number of patients with a megaloblastic anaemia no folic acid deficiency existed and our patients never

Table 6 *The cumulative excretion of isotope in urinary uric acid after oral gift of glycine ^{14}C before and during adenine administration*

	Re W ( )	Ru W ( )
7 days recovery of isotope in urinary uric acid		
without adenine	2 48	1 63
with adenine	1 53	0 72

showed any improvement in their megaloblastic anaemia after folic acid therapy it cannot be assumed that a folic acid deficiency is the only cause of this megaloblastic anaemia It is possible that because of the increased purine synthesis de novo in Lesch Nyhan patients a greater need for folic acid exists A justification for this is the discovery by Felix & De Mars that fibroblasts cultures from Lesch Nyhan patients have a strongly increased need for folic acid (4)

It seems probable that the disturbed nucleic acid synthesis in the erythrocytes which is the cause of the megaloblastosis is a result of the unbalanced formation of purine nucleotides

The disappearance of the megaloblastic anaemia by adenine therapy suggests that in this way the nucleic acid synthesis in the erythrocytes had been improved In the course of our investigations Felix & DeMars (4) published their findings that fibroblasts cultures from Lesch Nyhan patients did not grow in media to which no adenine or folic acid was added They also assumed that the increase in cell growth after addition of adenine is a result of an improved nucleic acid synthesis

During oral medication with adenine we noticed that the glycine ^{14}C incorporation in the urinary uric acid decreased This effect was not caused by the possible decrease of the kidney function as a result of the adenine administration The endogenous creatinine clearence and the concentrating ability are not changed under adenine treatment

The relationship between the deficiency of the enzyme hypoxanthine guanine phosphoribosyltransferase and the increased purine synthesis de novo could be found in a decreased feedback inhibition of the purine nucleotides as a consequence of the decreased resynthesis of these nucleotides On the other hand this relation could also be found in a greater availability of phosphoribosylpyrophosphate (P R P P) as a consequence of the enzyme deficiency which would result in an increase of substrate for the first rate limiting reaction in the purine synthesis de novo (20) a reaction catalysed by phosphoribosylpyrophosphate amidotransferase

The orally administered adenine could on the one hand consume the possible excess of P R P P by the conversion in adenosine monophosphate and on the other hand strengthen the feedback inhibition via the formed purine nucleotides In this way we can explain the decrease in the purine synthesis de novo we found in patients Re W and Ro W during adenine medication

The question is still open however whether through the decreased purine synthesis de novo the nucleic acid synthesis has improved The disappearance of the megaloblastic anaemia with adenine therapy at least suggests that at any rate this is the case in the erythrocytes The question which remains to be answered is whether the improvement of the nucleic acid synthesis also takes place in the cerebral ganglion cells and if this is the case it remains uncertain whether recovery of cerebral damage is possible in our patients

We continued oral adenine therapy and supplied allopurinol Up till now we did not see any change in the neurological symptoms in our patients probably because the cerebral damage was irreversible On the ground of our experience and the findings of Felix & De Mars it seems useful to treat patients with the Lesch Nyhan syndrome from birth with adenine and to add allopurinol to this medication to correct the hyperuricaemia and to prevent the formation of the renal toxic dioxyadenine from adenine by xanthine oxidase action (19)

SUMMARY

A report is given of the clinical and biochemical features of two brothers with the Lesch Nyhan syndrome A disappearance of the megaloblastic anaemia and a decrease of the purine synthesis de novo was found during oral adenine administration The influence of adenine medication on the disturbed metabolism in this syndrome is discussed Adenine in combination with allopurinol is suggested as a medication in the Lesch Nyhan syndrome



Fig 3 Bone marrow of Re W before *a* during *b* and 3 months after *c* termination of adenine administration

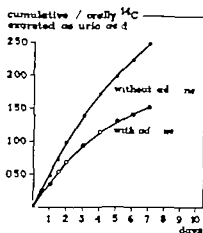


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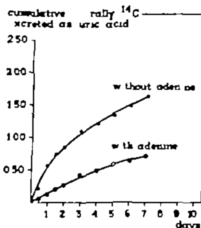


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PATTERNS OF MEDICAL AND SOCIAL RESEARCH IN PEDIATRICS

STEPHEN A RICHARDSON

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In pediatrics there is heavy emphasis both in training and research on the biology of bodily development, and particularly on pathological conditions in disease and disability Knowledge about the social conditions that influence children is largely at the common sense level enriched through experience into the art of clinical practice In the past this was justified by the absence of the social sciences but there is now a body of knowledge concepts and methods which can be brought to bear on social and bio social issues in pediatrics The purpose of this paper is to illustrate and discuss various ways in which social and medical scientists are working out productive ways of conducting bio social research in pediatrics

The first illustration deals with a problem identified in pediatric rehabilitation which gave impetus to a variety of studies by social scientists which have clarified the problem and provided some answers Important pediatric advances in the treatment of physically handicapped children occurred in the decade following World War II such as advances in medication bracing surgical correction physical and occupational therapy With these achievements there was also disappointment Disappointment as it was recognized that after intensive rehabilitation programs persons with physical disabilities were often dropping out of school early were having trouble finding jobs and losing their jobs even though they were technically able to perform the tasks

A British study of cerebral palsy (6) found

"The most striking feature shared to some extent by all those who require prolonged training is immaturity . . . It does seem however that these children have been denied experience in many activities, which given encouragement they could have enjoyed Many behave as though they had gone through their childhood on a conveyor belt system which involved no real responsibility or need for awareness of life around them (pp 93 94)

A study in the United States found (7)

"Cerebral palsied adults who are potentially employable and capable of social activity are typically unemployed and socially isolated" (p 633)

For the physician success is often measured by the improvement or disappearance of a patient's symptoms as seen during hospital or clinic visits These two follow up studies focus on the broader issue of the long term consequences of disability for later social functioning

The following questions are suggested by these studies

Are there certain social experiences that are essential for young people in order to develop the social skills needed for functioning as a mature adult? Do people with handicaps either miss or only partially share those experiences?

This paper is a revision of an invited paper given at the XII International Congress of Pediatrics Mexico 1968 under the title "Contributions of Social Science to Pediatrics"

REFERENCES

1 Catel W & Schmidt J Über familiäre gichtische Diathese in Verbindung mit zerebralen und renalen Symptomen bei einem Kleinkind *Deutsch Med Wschr 48* 2145 1959
2 Cartier P & Hamet M Les activites purine phosphoribosyltransferasiques des globules rouges humaines *Clin Chim Acta 20 205 1968*
3 Elkinton J R Huth E J Webster G D & McCance R A The renal excretion of hydrogen ion in renal tubular acidosis I quantitative assessment of the response to ammoniumchloride as an acid load *Amer J Med 29* 554 1960
4 Felix J S & DeMars R Purine requirement of cells cultured from humans affected with Lesch Nyhan syndrome (hypoxanthine guaninephosphoribosyltransferase deficiency) *Proc Nat Acad Sci 62* 536 1969
5 Kaufman J M Greene M L & Seegmiller J E Urine uric acid to creatinine ratio—a screening test for inherited disorder of purine metabolism *J Pediat 73* 583 1968
6 Kelley W N Seminars on the Lesch Nyhan syndrome Hypoxanthine guanine phosphoribosyltransferase deficiency in the Lesch Nyhan syndrome and gout *Fed Proc 27* 1047 1968
7 Kelley W N Greene M L Rosenbloom F M Henderson J F & Seegmiller J E Hypoxanthine guanine phosphoribosyltransferase deficiency in gout *Ann Intern Med 70* 155 1969
8 Labrune B Cartier P Hamet M Bonnenfaut F Velin J Ribierre M & Mallet R Encephalopathie familiale avec hyperuricemia *Presse Med 76* 2337 1968
9 Lesch M & Nyhan W L Familial disorder of uric acid metabolism and central nervous system function *Amer J Med 36* 561 1964
10 Liddle L Seegmiller J E & Laster L The enzymatic spectrophotometric method for determination of uric acid *J Lab Clin Med 54 903* 1959
11 Manzke H Hyperuricämie mit zerebral parese Syndrom eines hereditären purinstoffwechselleidens *Helv Paediat Acta 22* 258 1967
12 Marie J Royer P & Rappaport R Hyperuricemie congenitale avec troubles neurologiques renaux et sanguins *Arch Franc Pediat 24* 501 1967
13 Michener W M Hyperuricemia and cerebral retardation *Amer J Dis Child 113* 195 1967
14 Munsat T L Klinenberg J Carrel R & Menkes J Defect in purine metabolism and neurologic disease *Bull Los Angeles Neurol Soc 33* 101 1968
15 Nyhan W L Oliver W J & Lesch M A familial disorder of uric acid metabolism and central nervous system function II *J Pediat 67* [illegible] 1965
16 Nyhan W L Pesek J Sweetman L Carpenter D G & Carter C H Genetics of an X linked disorder of uric acid metabolism and cerebral function *Pediat Res 1* 5 1967
17 Nyhan W L Seminars on the Lesch Nyhan syndrome Introduction—clinical features *Fed Proc 27* 1027 1968
18 — Seminars on the Lesch Nyhan syndrome 1968
Summary of clinical features *Fed Proc 27* 10[illegible] 1968
19 Philips F S Thiersch J B & Bendich A Adenine intoxication in relation to in vivo formation and deposition of 2-8 dioxyadenine in renal tubulus *J Pharmacol Exp Ther 104* [illegible] 1952
20 Rosenbloom F M Henderson J F Caldwell H C Kelley W N & Seegmiller J E Biochemical bases of accelerated purine biosynthesis de novo in human fibroblasts lacking hypoxanthine guanine phosphoribosyltransferase *J Biol Chem 243* 1166 1968
21 Royer P *Actualites nephrologiques de l'hôpital Necker* Ed Flammarion Paris 1968 p 301
22 Seegmiller J E Rosenbloom F M & Kelley W N Enzyme defect associated with a sex linked human neurological disorder and excessive purine synthesis *Science 155* 1682 1967
23 Shapiro S L Sheppard G L Dreifuss F E & Newcombe D S X linked recessive inheritance of a syndrome of mental retardation with hyperuricemia *Proc Soc Exp Biol Med 1* [illegible] 1966
24 Zee S P M van der Monnens L A H Schretlen E D A M Een hereditaire purinestofwisselingsstoornis met een cerebrale aandoening en megaloblastaire anemie (Syndroom van Lesch en Nyhan) *Nederl T Geneesk 11* 14[illegible] 1968
25 Zee S P M van der Schretlen E D A M & Monnens L A H Megaloblastic anaemia in the Lesch Nyhan syndrome *Lancet 1* 1427 1968

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Cerebral palsied adults who are potentially employable and capable of social activity are typically unemployed and socially isolated (p 655)

For the physician success is often measured by the improvement or disappearance of a patient's symptoms as seen during hospital or clinic visits These two follow up studies focus on the broader issue of the long term consequences of disability for later social functioning

The following questions are suggested by these studies

Are there certain social experiences that are essential for young people in order to develop the social skills needed for functioning as a mature adult? Do people with handicaps either miss or only partially share these experiences?

This paper is a revision of an invited paper given at the XII International Congress of Pediatrics, Mexico 1968 under the title "Contributions of Social Science to Pediatrics"

Are there special difficulties or barriers which people with physical disabilities encounter in establishing social relationships with other people?

The following studies illustrate ways in which these questions have been explored

Kleck *et al* (8) designed an experiment to determine whether a person who is not handicapped behaves in a different way when he meets a person who is visibly handicapped compared to when he meets a person who is not handicapped The subjects of the experiment were high school students Each subject met a handicapped or non handicapped peer whom they had not previously known To insure that differences in the behavior of the high school subjects were due to the presence or absence of a physical handicap the experiment had to be designed so that apart from the presence or absence of a handicap the appearance manner and behavior of the handicapped and non handicapped peer were identical This was accomplished by the experimenters hiring and training a high school boy who acted as their confederate This confederate was not handicapped but could be made to appear either as a handicapped or non handicapped person This was done through the use of a wheel chair with a false bottom and by the confederate bending one of his knees and placing his leg below the knee into the false bottom This successfully created the illusion of a below knee amputation The confederate was trained to behave in exactly the same way toward every subject whether he appeared as handicapped or non handicapped Half of the subjects met one at a time with the confederate when he played the role of a normal person The other half met individually with the confederate when he played the role of a handicapped person None of the subjects knew of the experimental arrangements They all thought they were meeting another student subject

The following ideas were tested in the study

Many people experience some ambivalence of feelings when they first meet a handicapped person *This will make them fearful of spontaneity in case they reveal their negative feelings Meeting a handicapped person is also an* unfamiliar experience for most people From *this* reasoning it was predicted that a non handicapped person in meeting a handicapped person would be more stereotyped inhibited and overcontrolled and would be more anxious than in encounters with non handicapped persons

In many countries there is a strong value which dictates kind and considerate treatment of others especially when others are less fortunate than ourselves From this the investigators postulated that a non handicapped person will distort information in the direction he thinks the handicapped person would like to hear

The results showed that when the non handicapped subjects were in contact with the handicapped as compared with the non handicapped person they expressed opinions less representative of their beliefs This suggests that the handicapped person does not receive accurate or spontaneous feedback from others Absence of accurate feedback makes it difficult for the person who is handicapped to learn appropriate behavior and makes it difficult for him to develop social skills and know what others really think of him

The non handicapped subjects showed more emotional arousal as measured by the psycho galvanic skin response and were more formal and inhibited in their behavior toward the handicapped person This reduces the repertory of behavior to which the person with a handicap is exposed thus limiting the range of his experience in social relationships

A second experiment used a somewhat different approach (9) There are a wide range of social encounters in which the potential participants have some degree of choice as to whether to initiate or avoid a social encounter The choice will be influenced by the chooser's attitudes or values toward the person he may meet Essentially a value is a preference ordering among alternatives *The study of values*

was approached by using six drawings of the same child in which everything was held constant except the presence or absence of a visible physical disability and the type of disability. The pictures were laid before the child being tested and he was asked to look at each picture carefully. He was then asked "Which boy do you like best?" When the child indicated his preference this picture was then removed. He was asked again "Now which boy do you like best?" and the procedure was repeated until only one picture remained. In this way a rank ordering of each child's preferences was obtained.

The initial study was of 640 children aged 9–11 in the United States. The main findings were that the children preferred the non handicapped child to the five children with various handicaps. The same finding has since been obtained for children in Germany, Greece, Israel and Mexico. The children exhibited a remarkable degree of agreement in their preference order of the different handicaps. The order from most to least liked was: 1) A child without a visible handicap 2) A child with crutches and a brace on the left leg 3) A child in a wheelchair 4) A child with a left forearm amputation 5) A child with a slight facial disfigurement 6) An obese child. The children with physical handicaps that were tested displayed the same values as children who were not handicapped. A rather sad result.

These findings suggest that childrens' values towards disability will make them less inclined to initiate social relations with a handicapped than a non handicapped child. Another study of friendship patterns show handicapped children are less likely to be chosen as friends than non handicapped children (2).

These studies provide evidence suggesting the following answers to the general questions posed earlier:

Children with handicaps do miss or only partially share in certain social experiences that are essential to develop the social skills needed for functioning as an adult.

There are special difficulties or barriers which children with physical disabilities encounter in establishing social relationships with others.

These and other studies (10) suggest that it is as important for a handicapped child to receive especial training in social skills as it is for him to receive the more traditional rehabilitation services which have focused on bodily pathology. There have, of course, been recreational programs planned for handicapped children, but these have been hampered by lack of specific knowledge of the kinds of experiences the children have had, the kinds of skills they most need to learn, the techniques for dealing with the social obstacles they will encounter because of their disability, and how these skills are best taught. While knowledge from social science studies is still fragmentary and partial, it is growing quickly. There are social rehabilitation techniques that could now be usefully employed, e.g. some specific techniques that are used by handicapped persons in "breaking through" the social barriers created by their disabilities (3), and these could be taught using role playing and other training techniques.

A second illustration of bio social research is a finding of a medical epidemiologist that raises a challenging question for social scientists. Gruenberg (5) examined the prevalence of mental subnormality at different age levels. Despite the differences found in various studies, there was agreement on one striking finding:

> After the age of fourteen the prevalence is rarely half as high as at the age of fourteen. ... For this to occur a large group of people regarded as retarded at fourteen must improve in their functioning to the point where people no longer regard them as retarded and also succeed in escaping their history of earlier unsatisfactory performance.
>
> Either these individuals are continuing to be extremely handicapped in later life and are unknown because the services they need are unavailable to them ... or they have stopped being retarded in any real sense at all and do not need any special protection, help or services, in which case one had better change one's concept of what real mental retardation really is (pp 273 274).

A study by Edgerton (4) throws some light on the question. He followed up 48 adults who

had been inmates of a large state institution for the mentally retarded, and had been discharged having been judged able to conduct their lives in the community without supervision Their mean IQ was 66 with a range from 48–84 Using intensive interviews Edgerton obtained a detailed picture of their day to day lives and of some of the difficulties they en countered

The critical problem the ex patients faced after release from the hospital was the conti nued attempt to hide their incompetence from others and to try to pass as normal people Ex patients enter the outside world without any of the possessions which normal people accumulate To the ex patients these possessions are essential symbols of being normal in the outside world They try to remedy this by acquiring souvenirs photo albums and odd ments from junk shops trash cans and friends In addition to fabricating a past to conceal their real history they acquire new souvenirs of their lives after release and display them proudly Although they cannot read ade quately they buy books and magazines and display them prominently in their homes Because they receive little or no mail they try and give the appearance of doing so by keeping and displaying what they do receive and sometimes collecting other people s discarded letters

In city life there are a variety of situations where everyone is called upon to read—using telephones bus destinations shopping etc The expatient develops excuses for hiding his incompetence in reading to others e g he says to a normal person that he s forgotten his glasses and can t see the words in question (p 164) The obliging normal then reads for him

The use of numbers presents a difficult chal lenge For example telling time One device used is to wear a watch that is not running They can then ruefully look at their watches and say My watch stopped But to ask What time is it? can lead to replies they can not understand such as It s twenty to nine or It s eight forty To avoid confusion a number of techniques were used, such as ask ing in numbers they could understand, such as Is it nine o clock yet?

One essential component for coping with the problems of living in the community is having a benefactor —a normal person who helps them cope with everyday problems helps them hide their areas of incompetence and avoid getting into trouble Various people such as landladies neighbors, employers and spouses acted as benefactors The majority knew the ex-patient s past, and helped with problems of biography management and concealment of the past They helped conceal deficits in everyday competence such as appearance dress or speech and inability to read write or deal with numbers

Another critical problem for the ex patients was the building up and maintenance of their self esteem However well the ex patients de velop such devices to pass as normal people they cannot fail to realize that their compe tence in many aspects of everyday life is clearly less than that of the normals with whom they must associate Such a realization is potentially devastating to their self esteem and if the in tegrity of the self is to be maintained imputa tions of stupidity must be denied The process of denial is continuous (p 169) Many of them appear to believe that they are rela tively less competent than normal people be cause they have suffered the depriving experi ence of having been confined—*wrongly* of course—in Pacific State Hospital (p 169) In the ex patients efforts to establish their worth as normal human beings *they* are greatly in need of the affection and respect of normal persons (p 201) Benefactors often help to supply this want In order to convey affection without seeming to be patronizing the normal benefactor must be highly sensitive to the expatient s need for self respect (p 201)

Edgerton also brings out the terrible stigma which the term mentally retarded imposes on those who are so designated by the society This stigma has been imposed and reinforced

from childhood on Prior to being placed in the State Hospital Edgerton's subjects had been repeatedly told directly or indirectly that their intelligence was deficient Parents peers teachers neighbors and even strangers presented a consistent refrain of rejection and humiliation (p 146) The patient was then "accused and found guilty of being so stupid that he was considered incompetent to manage his own life (p 145) and was committed to a mental hospital

It is clear there are services that these ex patients need The dilemma is how can these services be provided on an organized basis without stripping away the disguise these mentally retarded adults so struggle to put on and maintain in order to hide their incompetence pass as normal and maintain some self-esteem In the future this dilemma can only be resolved if we reduce the frequency with which mentally subnormal children and youth experience failure rejection and humiliation This requires a careful rethinking of present services for children and youth For example the peak of prevalence for mental subnormality found in the last years of school strongly suggests that the intellectual demands made on young people are greater than those that many of them will ever again have to face We need to consider the cost of frequent failure in day to day school life for large numbers of children and what this does to their self-esteem and future development

The two illustrations given are concerned with issues where further understanding is greatly facilitated by closer communication and working together of pediatricians social scientists and educators One of the serious obstacles to cooperation between the social and medical sciences has been their isolation one from the other with little common training communication or mutual education The lack of close working relations between the social and medical sciences is most serious in dealing with the numerous problems which concern pediatricians which neither the biological nor social scientists can tackle fully alone but demand both sets of research skills For example childhood accidents which are one of the major causes of morbidity and mortality the causes and the consequences of malnutrition mental subnormality poverty the battered child syndrome and the child who has educational handicaps

A community study of mental subnormality will be used as an illustration of bio social cooperative research (1)

A goal of the study was to explore the relative contribution of social biological and genetic factors to the causes of mental subnormality and its subtypes One step toward this goal was to examine the kinds of families where there are mentally subnormal children The children studied were the total population of mentally subnormal children aged 8–10 resident in Aberdeen Scotland From the total population of 104 children there were 6 where the causes had been clearly identified—5 with a known genetic origin—Down's syndrome—and one child where the subnormality was clearly the consequence of severe meningoencephalitis in the third year of life

The families of the remaining 98 children where the causes of M S were unclear were classified by the occupation of the father or head of the household The occupations were then grouped into the broad social class classifications used by the Registrar General The children were grouped into subtypes based on IQ score and the presence or absence of clinical evidence of CNS damage For the M S children having IQs of less than 50 and clear evidence of CNS damage there are sound grounds in the clinical findings of brain damage for believing that the mental subnormality derived primarily from CNS damage These children are randomly distributed across social classes (Table 1) This provides no reasonable basis for inferring that social environment or familial constitutional factors have made a significant contribution to the production of the handicap

The least severely subnormal children are those with IQ of 60 or greater and without

had been inmates of a large state institution for the mentally retarded and had been discharged having been judged able to conduct their lives in the community without supervision Their mean IQ was 66 with a range from 48–84 Using intensive interviews Edgerton obtained a detailed picture of their day to day lives and of some of the difficulties they encountered

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Edgerton also brings out the terrible stigma which the term mentally retarded imposes on those who are so designated by the society This stigma has been imposed and reinforced

normality are exposed to a combination of maternal obstetrical and social sub-optimal conditions in their early development. Etiology is more likely to derive from some combination of social-environmental and familial constitutional factors existing in conjunction with ill defined reproductive inadequacy rather than from any definite obstetrical risk condition for CNS insult. Apart from the initial identification of 5 cases with known genetic causes there was no way in the design of the study to fully distinguish between genetic and social environmental etiology.

These examples of research illustrate ways social and medical scientists can cooperate in pediatrics. One approach has been the selection by social scientists of questions raised by physicians which can be taken up and effectively pursued using the special skills of the social scientists, a body of related knowledge, a pertinent conceptual framework, and special methods for studying social phenomena. There are some problems in pediatrics where social scientists can work effectively providing pediatricians contribute some minimum cooperation and goodwill. But there are many research problems in pediatrics where more intensive and continuous cooperation is necessary between social and medical scientists, and where they must become full working partners. In these cases each must fully contribute his special knowledge and skills to the formulation, course and results of the research so that the product will be different from what either could achieve alone. This form of cooperation in research often takes years to evolve between the research partners, requiring a mutual education to find out how best to use their respective research and writing skills.

The pediatrician has settings and opportunities in the hospital, his office and on house calls to gain access to behavior of the neonate, child and adolescent and to some extent to his patients' families. This range of behavior in health and more particularly during illness and disability provides valuable opportunities for observations and insight, from which the social scientist can gain. Once a question has been raised, however, the pursuit of the question may take the social scientist into social settings rarely seen by the physician—into the classroom, the playground, the summer camp. Just as the pediatrician can enrich the experience and research opportunities of the social scientist, so the social scientists can also help the pediatrician. The social scientist through differences in viewpoint and in the settings in which he works can raise research issues that may not have occurred to the pediatrician, questions about medical practices which have become habitual and which may no longer serve an appropriate function.

Starting with the great bacteriological discoveries of the nineteenth century medicine sought specific causes for specific disorders. This single factor approach to research then came to be seen as inadequate. There is a growing recognition that more complex multifactorial conceptual models are necessary, and that many diseases and disabilities are the outcome of a complex process over time involving biological, genetic and social environmental components.

In summary, there are two broad categories of research in which the social and medical scientist must work together. One is in furthering understanding of the bio-social factors which contribute to the causes and consequences of disease and disability. The other is in the planning, conduct and evaluation of health services.

The long and almost complete separation of the social and medical sciences is being complemented with increasing speed by various forms of cooperation and collaboration. These forms of bio-social research are essential to obtain the knowledge necessary to take seriously the goal of the World Health Organization as reflected in their definition of helath, "a state of complete physical, mental and social well being, as well as being the absence of disease or infirmity".

Table 1 *Social class distribution of mentally subnormal children with I Q <50 and clinical evidence of central nervous system damag*

Social class	Actual no	Expected no	Actual no / Expected no [b]
I–IIIa (2405)	7	6 80	1 03
IIIb (1904)	4	5 41	0 74
IIIc (1648)	6	4 70	1 28
IV (1087)	3	3 10	0 97
V (1044)	3	2 90	1 03
Total (8088)	23	23	1 00

Total at risk in social class × Total M S <50 with C N S damage/ Total at risk in total population
[b] A figure greater than 1 0 indicates a higher concentration and a figure less than 1 0 a lower concentration of cases than expected

evidence of CNS damage It is *this subtype where the literature abounds in diverse opinions about etiology, because at present there is no clear evidence for attributing cause to any one of the general etiological factors* There are no children with *this least severe* subtype of subnormality among the upper social class families—where the father is in a non manual journeyman or artisan occupation (Table 2) By contrast these children are heavily overrepresented in the semi skilled manual and especially in the unskilled manual occupations *the lowest social classes*

Social class is a very general indicator for characterizing the life styles of families To learn more about the lower class families where the least severe subtype of subnormality is so heavily concentrated a number of additional family characteristics were examined

Table 2 *Social class distribution of children with I Q ≥60 without clinical evidence of central nervous system damage*

Social class	Actual no	Expected no	Actual no / Expected no
I–IIIa (2405)	0	11 3	—
IIIb (1904)	0	8 9	—
IIIc (1648)	8	7 7	1 0
IV (1087)	12	5 1	2 4
V (1044)	18	4 9	3 7
Total (8088)	38		

The families in the lowest social classes with children who are minimally subnormal are overrepresented when the following features are present *1) Five or more children 2) Residence* in a poor housing area 3) Living under more crowded conditions as measured by person room ratio 4) Where the mother held a semi- or unskilled occupation prior to marriage 5) A higher frequency of siblings that are mentally subnormal compared to families of the same social classes where there is not an 8–10 year old M S child

These family characteristics are not, of course factors which cause minimal subnormality Rather they characterize the kinds of families where there is a higher risk of minimal subnormality Knowing these characteristics provides a basis for further more intensive studies on a carefully defined population The results also provide a basis for speculative inference suggesting guilt by association On in*ferential grounds there is evidence to support* the conclusion that social environmental factors are contributing to this least severe clinical subtype of M S

For the same group of minimally subnormal children the obstetrical and perinatal histories *of the mother and infant were compared* with a comparable population of children who were not mentally subnormal

In complications of pregnancy the mothers of the minimally subnormal children did not experience severe or moderate preeclamptic toxemia more often than mothers of the com*parison population* but did have a higher frequency of antipartum hemorrhage and threatened abortion when both types of bleeding are combined There were no significant differences in either duration of labor or in types of presentation or delivery There was a higher proportion of minimally subnormal children with birth weights under 6 lbs and gestational ages of 37 weeks or under A higher proportion of the mothers of these children were 5 ft *in height or under*

These findings suggest that these children in the mildest clinical subtype of mental sub-

normality are exposed to a combination of maternal obstetrical and social sub-optimal conditions in their early development Etiology is more likely to derive from some combination of social-environmental and familial constitutional factors existing in conjunction with ill defined reproductive inadequacy rather than from any definite obstetrical risk condition for CNS insult Apart from the initial identification of 5 cases with known genetic causes there was no way in the design of the study to fully distinguish between genetic and social environmental etiology

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In summary there are two broad categories of research in which the social and medical scientist must work together One is in furthering understanding of the bio social factors which contribute to the causes and consequences of disease and disability The other is in the planning conduct and evaluation of health service

The long and almost complete separation of the social and medical sciences is being complemented with increasing speed by various forms of cooperation and collaboration These forms of bio social research are essential to obtain the knowledge necessary to take seriously the goal of the World Health Organization as reflected in their definition of helath "a state of complete physical mental and social well being as well as being "the absence of disease or infirmity

REFERENCES

1 Birch H G Richardson S A Baird D Horobin G & Illsley R *Mental subnormality—a community study of children* Williams and Wilkins Co Baltimore In Press
2 Centers L & Centers R Peer group attitudes toward the amputate child *J Soc Psychol 61* 127 1964
3 Davis F Deviance disavowal and normalization *Social Problems 9* 120 1961
4 Edgerton B *The cloak of competence* Univ of California Press 1967
5 Gruenberg M Epidemiology *in* H A Stevens & R Heber (eds) *Mental retardation* Univ of Chicago 1964
6 Ingram J E *Living with cerebral palsy* Studies in Medicine No 14 The Spastics Society in association with Wm Heinemann (Medical) Books Ltd London 1964
7 Klapper Z S & Birch H G The relation of childhood characteristics to outcome in young adults with cerebral palsy *Develop Med Child Neurol 18* 645 1966
8 Kleck R Ono H & Hastorf A H The effects of physical deviance upon face to face interaction *Human Relations 19* 425 1966
9 Richardson S A Goodman N Hastorf A H & Dornbush S M Cultural uniformity in reaction to physical disabilities *Amer Sociol Rev 26* 241 1961
10 Richardson S A The effects of physical disability on the socialization of a child *in* D A Goslin (ed) *Handbook of socialization theory and research* Rand McNally and Co Chicago 1969

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INTESTINAL MALABSORPTION A CLINICAL STUDY OF 22 CHILDREN OVER 2 YEARS OF AGE

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A classic clinical picture and steatorrhoea with gluten intolerance are the criteria usually applied in the diagnosis of coeliac disease Hence it is difficult to detect the existence of atypical symptomatology in the disease Nevertheless more or less atypical forms have been described by many authors especially in older children (2 3 8) When the finding of villous atrophy of the intestinal mucosa is taken as the diagnostic criterion of coeliac disease it is possible to evaluate the clinical manifestations in coeliac disease and even detect other conditions associated with intestinal mucosal villous atrophy

This report presents the clinical symptomatology and findings in respect of 22 children over 2 years of age in whom significant villous atrophy was found The authors believe that these patients are all suffering from coeliac disease although this name has not been applied because the diagnosis has not been properly verified in each case We have previously presented a series of infants with similar findings (11)

PATIENTS

The patients in the series were children over 2 years of age with partial or subtotal villous atrophy of the duodenojejunal mucosa The series comprised all such cases observed during a 7 year period (1962 1969) at the Children's Hospital University of Helsinki (admission rate approximately 7000/year) Fig 1 indicates the age of the patients on admission During the same 7 year period in which these patients were found villous atrophy was also verified in 110 infants under 2 years of age

Biopsy was performed in cases in which the malabsorption syndrome was suspected on the basis of the clinical picture and the results of laboratory tests

Of the 22 patients 15 were female such a preponderance of females has been noted in some other series (1) but not in all (3)

The incidence of coeliac disease and of diabetes mellitus were higher among the relatives of these 22 patients than in the general population Of the 73 siblings two suffered from coeliac disease and two from diabetes mellitus In addition two had a severe allergic disorder All the 44 parents were healthy in these respects but in seven families there was diabetes mellitus and in one family coeliac disease in other relatives The birth weights of the patients were normal and weaning had usually taken place between 2 weeks and 4 months Four patients however were breast fed for more than 6 months and 2 patients even up to the age of 1 year

METHODS

The laboratory examinations reported were performed by the routine methods we have described earlier (10) The biopsies were made by multipurpose suction biopsy tube and with a pediatric Crosby capsule from the distal duodenum or the proximal jejunum The biopsies were evaluated by conventional light microscopy and the villi were measured (5) The following classification was adopted normal—median villous length exceeding 300 μ slight changes—length 250–300 μ partial villous atrophy (PVA)—length 150–250 μ subtotal villous atrophy (SVA)—length under 150 μ In addition observation was made on the epithelial cell layer and cell infiltration in the lamina propria

SYMPTOMS AND SIGNS

In 15 patients the first gastrointestinal symptoms had occurred before the age of 2 years (Fig 1) These initial symptoms were prolonged or severe watery diarrhoea sometimes

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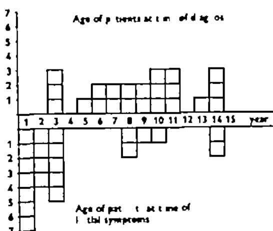


Fig 1 Ages of patients at time of diagnosis and at time of onset of initial symptoms Diagnoses made before 2 years of age are not included in this series

pale and bulky stools and failure to thrive Coeliac disease was suspected in some cases, although no definitive diagnosis was made However these initial symptoms subsided more or less completely in 14 cases, and in consequence the diagnosis was postponed In 5 cases the apparent cause of this remission was short term treatment with a gluten free diet whereas in the others it occurred spontaneously Table 1 indicates the presenting symptoms In 7 cases suspicion was aroused at a routine check by the school physician or on some similar occasion Table 2 presents all the symptoms observed by the patients or their parents

Table 1 *Main symptoms which drew the attention of the parents (A) or the physician (B) to the illness*

	No of cases
A First symptoms observed by the parents	
Abnormal stools and retarded growth	3
Abdominal pain	2
Anorexia	2
Convulsive seizures	2
Diarrhoea and tetany	1
Diarrhoea and haemorrhage	1
Diarrhoea and abdominal pain	1
Abdominal pain and tetany	1
Vomiting	1
Lassitude	
B First symptoms found by a physician at the child welfare clinic or on school examination	
Retarded growth	4
Pallor anaemia	3

Table 2 *Symptoms observed by the patients themselves or by their parents*

Symptoms	No of patients
Psychic tiredness and/or irritability	13
Anorexia	9
Abdominal pain	8
Pale bulky and offensive stools	8
Retarded growth	7
Vomiting	6
Failure to gain weight	5
Watery diarrhoea recurrent	5
Pallor	4
Constipation	4
Abdominal distention	3
Headache	3
Dislike of wheat	3
Tetanic cramps	2
Oedema	2

and in a single case haemorrhage rectal prolapse nausea hypertrichosis pica conjunctival irritation melaena sensitivity to infections and loss of hair

These results demonstrate the relative rarity of the typical coeliac picture the great variability of the symptoms, and the existence of cases with very few and subjectively minimal symptoms

Three of these 22 patients suffered concomitantly from diabetes mellitus and in addition one of them from autoimmune thyroiditis The chronological relationships of these diseases are indicated in Table 3 In addition one of the patients suffered from a convulsive disorder with intracranial calcification suggesting the Sturge–Weber syndrome one suffered from Besnier's prurigo and in one case a γA globulinaemia was found at the same time as the intestinal disease

As a rule the clinical findings on admission were rather sparse Retardation of growth was noted in every case (Fig 2) However 10 patients were within two standard deviations of the mean in both height and weight although their growth curves exhibited a chance of pattern The heights were more often subnormal than the weights The abdomen was somewhat enlarged in 13 cases but only in 6 cases markedly so Haematomas were found in 1 patient and emphysematous chest in 2 Diar

Table 3 Chronological order of the onset of main symptoms and diseases in three patients suffering concomitantly from intestinal disease and endocrinopathies

Patient	Age at onset of first intestinal symptoms	Age at diagnosis of		
		Malabsorption	Diabetes mellitus	Thyroiditis
M H	2 y 6 mo	10 y 2 mo	1 y 9 mo	10 y
H T	under 1 y	9 y 3 mo	8 y 6 mo	
H N	1 y 2 mo	10 y 8 mo	1 y 4 mo	

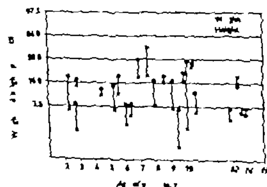
rhoea was observed in 4 cases on admission and constipation in 3 In all the other 15 cases the frequency of stools was normal the quality of stools however was not investigated systematically In one case convulsions were attributable to hypoglycaemia and in another to concurrent brain disease (Sturge–Weber)

LABORATORY EXAMINATIONS

Th results of laboratory X ray and histopathological examinations are presented in Table 4 and Fig 3 As was expected the faecal fat excretion was almost constantly increased (Fig 3) The D-xylose test was abnormal however in about 50 of cases only In particular all the diabetics had a normal excretion of D-xylose

The results of laboratory tests demonstrated that false negative results were derived from every test employed for the indication of malabsorption viz faecal fat the D xylose excretion test FIGLU test precipitins to cow s milk and gluten and barium meal Nevertheless every patient had an abnormal result in at least two of these tests

One group of examinations was concerned with investigation of the nutritional defects of the patients Anaemia in particular iron-deficiency anaemia was the commonest nutritional defect after retarded growth In some cases it was very severe (Fig 3) In four cases megaloblastic features were combined with the iron deficiency In every case this was due to



F g 2 Weights and heights of patients at the time of admission

Table 4 Laboratory X ray and histopathological findings

Since not all the patients were successfully examined with all the tests the numbers of those examined are indicated All tests except biopsy were made before treatment was started

Faecal fat excretion more than 5 g day	18 22
Fat absorption less than 90	17 21
Abnormal fat excretion and/or absorption	19 22
D-xylose excretion less than 20	11/21
Urinary FIGLU positive	17/20
Haemoglobin less than 10 g/100 ml	12/22
Haemoglobin less than 11 g/100 ml	19 22
Serum iron less than 40 μg 100 ml	16 [illegible]
Megaloblastic features in bone marrow or peripheral blood	4/22
Prothrombin less than 80	7 19
Serum calcium less than 4 mEq/l	2/17
Serum alkaline phosphatase activity more than 10 B L units	2/17
Generalized aminoaciduria	2/20
Normal sweat chlorides	20 20
Serum total protein less than 6 5 g/100 ml	6/16
Decrease of serum IgA during treatment	5 1[illegible]
Absent serum IgA	1/22
Precipitins to cow s milk	15/18
Precipitins to gluten	9/18
Barium meal malabsorption pattern	15/16
Bone X ray retarded bone age	11/11
Duodenojejunal biopsy	
Subtotal villous atrophy	17/22
Partial villous atrophy[a]	5/22

[a] Three of these were not successfully biopsied until 1 2 years after the start of therapy

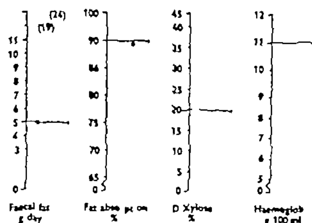


Fig 3 Results of determination of faecal fat excretion estimation of fat absorption D-xylose tests and determinations of blood haemoglobin

folic acid deficiency (according to the measurement of serum folic acid and vitamin B_{12} in these cases) The other nutritional defects clinically manifest were hypocalcaemic tetany (in two cases) rickets (in two cases not the same as the preceding) hypoprothrombinaemic haemorrhage (in one) hypoglycaemic convulsions (in one) and hypoproteinaemic oedema (in one)

As a rule, intestinal biopsy showed a typical flat mucosa with round cell infiltration and an abnormal epithelial cell layer Two of the patients with partial villous atrophy had not undergone any preceding therapy

RESULTS OF TREATMENT

For study of the possible role of iron deficiency in mucosal damage (7) 2 patients (patients

Table 5 *Results of treatment with gluten free diet in 20 patients*

In 3 cases (A M P M L & L O) two separate periods of treatment were concerned

Duration of diet		Responses to diet[a] Growth	Absorption	Mucosa	IgA
Strictly gluten free diet					
I I	8 mo	+	NS	NS	–
S L P	1 y	+	NS	NS	+
A M P diet 2	1 y	+	NS	NS	
R R	1 y 1 mo	+	NS	NS	
K S	1 y 10 mo	+	NS	NS	–
M U	2 y 5 mo	+	+	±	–
H K	2 y 7 mo	+	+	–	Absent
S K	3 y	+	+		NS
H T	3 y 9 mo	–	+	–	+
T M	4 y 1 mo	–	–	–[b]	NS
(J P	3 y 5 mo)[d]	+	±	±	NS
J P	5 y 10 mo	+	+	–	NS
Poorly controlled gluten free diet					
J F	2 y 1 mo		±	–	–
M L diet 2	2 y 7 mo	+	±	–	–
L O diet 2	3 y 4 mo	+	–	–	
T K	3 y 7 mo	–	+	–	–
S E	3 y 10 mo	+	+	–	
Gluten free + unrestricted diet					
M L diet 1	1 y 0 mo 2 y 4 mo	–	NS	NS	NS
A H	2 y 0 mo + 0 y 4 mo	+	–	–	
F K	2 y 0 mo + 0 y 6 mo	+	–	–	–
H N	5 y 5 mo + 0 y 1 mo		+	–[b]	NS
A M P diet 1	3 y 2 mo + 2 y 1 mo	+	–	–	NS
L O diet 1	0 y 6 mo + 2 y 3 mo	+	–	–	NS
M H	3 y 0 mo + 0 y 7 mo	+	–	–[b]	

[a] Explanations of symbols in table + = full normalisation ± = improvement – = no change NS = not studied
[b] Initial biopsy failed
Hypothyroid patient treated with thyroidin
[d] The patient J P was investigated twice after periods of different lengths

(U and S E in Table 5) with late onset of the disease no typical coeliac symptoms and severe iron-deficiency anaemia were initially treated with iron alone for 3 years and 6 months respectively and subsequently re-examined No improvement in absorption function or mucosal damage was observed

Treatment with gluten free diet (rice and corn being the only cereals allowed) was then started in every case Table 5 presents the results obtained Follow up examinations were made in 20 cases however in 1 patient with hypothyroidism induced by thyroiditis (M H) no conclusion could be drawn in view of the impracticability of distinguishing the effects of the gluten free diet and the thyroxin given simultaneously In others (19 cases) a good clinical response especially in regard to growth was found in 17 cases improvement in absorptive function in 11 of the 16 cases tested and improvement in the intestinal mucosa in 3 cases out of 13 Some response in respect of either growth absorptive function or mucosa was found in all 19 cases

The patients on a strictly gluten free diet invariably displayed a clinical response normalisation of the absorptive function and in some even normalisation of the mucosa although not until treatment had been continued for 2-5 years Serum IgA showed a decrease in many patients Precipitins to gluten disappeared in all but 1 case but precipitins to cow's milk persisted in 4 of the 10 patients who were initially found to have precipitins

The patients on a poorly controlled gluten free diet although with restricted gluten were all also in good clinical condition growing well and even exhibiting improvement in the absorptive function The mucosal damage did not heal in this group and the serum IgA level remained unchanged Precipitins to gluten were also found during treatment in 1 patient

The third group includes cases in which the patients were initially on a gluten free and then on a more or less normal diet All except 1 patient showed initially a response in growth but premature discontinuation of dietary treatment arrested the progress towards recovery The final results were dependent on the length of the period of gluten-containing diet All 3 patients kept on a gluten-containing diet for more than 2 years suffered clinical relapse The clinical relapses were manifested by a change in the growth pattern In addition 1 patient had convulsions of unknown etiology (hypoglycaemia?) The serum IgA was high in all three during the relapse and precipitins were found Two of the others displayed an increase in serum IgA and an increase in precipitins some months after the start of the unrestricted diet In 1 case these immunological variants were followed when clinical relapse was developing (Table 6)

Table 6 *Development of serum IgA concentration and precipitins to cow's milk and to gluten when a relapse was developing in a patient (A M P)*

The patient was initially treated with a poorly controlled gluten free diet for 3 year and 2 month with a rather good clinical response Then 2 year 1 month before the start of a new diet period the patient was started on an unrestricted diet A change in the growth pattern and anorexia were the only clinical symptoms In the table the time of beginning a new gluten free diet is denoted by 0

	Time			
	−2 y	4 mo −1 y	0	+3 mo
Serum IgA mg/100 ml	22	90	126	16
Precipitins to cow's milk		+		−
Precipitins to gluten	−	−	+	−

COMPARISON OF MANIFESTATIONS AND RESPONSES TO THERAPY IN PATIENTS OF DIFFERENT TYPES

It was found that the patients with diabetes mellitus and a γA globulinaemia all had some typical coeliac symptoms Absorption tests revealed clear malabsorption except that the diabetics had a normal D xylose test The diabetics responded rather slowly to the diet and normalisation of the mucosa was not observable in these patients but the diet was strict *in* only 1 patient out of 3 However the patient with

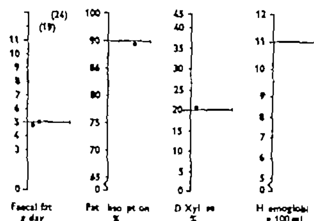


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a γA-globulinaemia responded completely to the gluten free diet

Among the patients without any significant concomitant disease two main groups can be distinguished 11 patients had displayed gastrointestinal symptoms even during infancy (early onset group) and in 7 the onset occurred after 2 years of age (late onset group) In the former group gastrointestinal symptoms were rather common subsequently Nevertheless three of these patients were later completely without gastrointestinal symptoms as were also 6 of the 7 patients with late onset Retardation of growth was more pronounced in the patients with early onset along with various nutritional defects, except for iron deficiency and megaloblastic anaemia which were again commoner or more severe in the late onset group The results of absorption tests were similar in the two groups The 2 patients who primarily (without any previous therapy) had only partial villous atrophy belonged to the late onset group No conclusions can be drawn in regard to the responses to therapy in the different types of disease

DISCUSSION

The first question which may arise from this report is whether these patients were suffering from coeliac disease The symptomatology was certainly not classic in nature some signs of malabsorption were discovered in every one and a favourable response to a gluten free diet was observed in all the 19 cases in which a proper follow up was possible, but the permanency of the gluten intolerance was not tested systematically If this satisfies the criteria of coeliac disease then the patients may be termed coeliacs The authors are of the opinion that they are all in fact coeliacs although it must be admitted that this was not completely confirmed

The origin of diabetic enteropathies has often been discussed (4 9) The chronological relationships of the diseases (Table 3) the findings and the responses to the diet suggest that in the present series of patients the enteropathy is not a complication of diabetes, but more probably an independent disease, coeliac disease, which for some reason combines with diabetes more often than could be expected from chance alone It should also be noted that diabetes was commoner in the sibs and other relatives of these non-diabetic patients than would be anticipated The patient with a γA globulinaemia had typical coeliac disease with confirmed gluten intolerance

Gluten intolerance (coeliac disease) usually becomes manifest some months after the introduction of cereals into the diet Consequently the diagnosis is usually made before the child reaches the age of 2 years Why then was the diagnosis made so late in this group of patients? The different reasons for this can be enumerated as follows

1 early onset, but spontaneous remission of symptoms for some years (9 patients)

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4 late onset of the disease (7 patients)

From the practical standpoint it is very important to note that the actual symptomatology of patients in this age group is usually atypical retardation of growth and anaemia often being the only symptoms The retrospective data concerning stools are very unreliable Moreover real diarrhoea is often lacking and the patients may even be constipated Abdominal pain is another symptom worth noting Nutritional defects are further important diagnostic indications in fact we have found that coeliac disease is the commonest cause of severe iron deficiency megaloblastic anaemia and deficiency rickets in children over 2 years of age

The results of absorption tests demonstrate that at least as far as those applied in this study are concerned no single one alone would pro-

the correct answer in all patients. This is in conformity with the observations of some other research workers (6). Apparently the most reliable single test is that based upon faecal fat. As the authors have suggested earlier (...) a considerably higher degree of reliability in detecting malabsorption can be achieved by means of a multiple test pattern. However the results of this report emphasize the importance of biopsy for final diagnosis, particularly in atypical cases.

Very few conclusions can be drawn from the results of treatment. It is evident that maintenance of a strictly gluten free diet is extremely difficult in this age group, and thus an objective method for checking observance of the diet is needed. The finding of precipitins to gluten is practicable in many cases, but unfortunately does not detect all. Marked elevation of the serum IgA level and reappearance of precipitins herald a clinical relapse.

The results of treatment demonstrate that restriction of gluten leads to clinical improvement and better absorptive function, but total exclusion of gluten is necessary for normalisation of the mucosa, which in any event is a very slow process. In every case in this series resumption of a gluten-containing diet, even if somewhat restricted, induced a clinical relapse after some years.

SUMMARY

This paper reports on the clinical picture and findings in 22 children over 2 years of age in whom the malabsorption syndrome was suspected and significant intestinal villous atrophy was found. Gluten intolerance was demonstrated in every one of the 19 patients in whom the results of treatment were followed. The authors consider that all the patients of this series were suffering from coeliac disease. Three of the 22 patients had concomitant diabetes mellitus and one of these additionally autoimmune thyroiditis. One patient had a-γA globulinaemia. The correlations of these diseases are discussed.

Although the diagnoses in these cases were made when the patients were over 2 years old, in 15 cases the first symptoms had been seen during infancy. The main reasons for the postponement of diagnosis were spontaneous remission of the initial symptoms, amelioration of the symptoms on treatment, and in some cases actual late onset of the disease. The classic clinical picture of coeliac disease was rare, and in many cases, especially those in which the symptoms started late, the condition was only manifested by a few symptoms: retarded growth and anaemia. The results of absorption tests indicated that no single test is absolutely reliable for detecting malabsorption, but that application of multiple tests increases the reliability. The laboratory tests revealed many nutritional defects, the commonest of which was iron deficiency. Stress is laid upon the importance of biopsy for confirming the diagnosis. The results of treatment demonstrate that the restriction of gluten induces clinical and absorptive improvement, but normalisation of the villous structure was achieved only by strict elimination over a period of many years, if at all. Patients with a relatively or totally unrestricted diet all relapsed after some years. Elevation of the serum IgA level and reapperance of precipitins preceded the relapse.

REFERENCES

1. Buchan D J & Gerrard J W. Celiac disease. Problems in diagnosis and effects of a gluten free diet. *Ann Intern Med 57* 85 196..
2. Gerrard J W & Lubos M C. The malabsorption syndromes. *Pediat Clin N Amer 14* 73 196.
3. Hamilton J R, Lynch M J & Reilly B J. Active coeliac disease in childhood. *Quart J Med 38* 135 1969
4. Hooft C, Devos E, Kriekemans & Damme.. van J. Malabsorption and diabetes mellitus in children. *Helv Paediat Acta 23* 478 1968
5. Kaufmann P. Duodenojejunal histology in malabsorption syndrome in infants. *Ann Paediat Fenn 12* 101 1966
6. McNeish A S & Willoughby M L N. Whole blood lactase as a screening test for coeliac disease in childhood. *Lancet 1* 442 1969
Naiman J L, Oski F K, Diamond C K, Vawter C F & Schwachman H. The gastrointestinal effects of iron deficiency anemia. *Pediatrics 33* 83 1964

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FIBRINOGEN AND FIBRINOLYSIS IN THE RESPIRATORY DISTRESS SYNDROME OBSERVATION DURING THE FIRST DAY OF LIFE

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The relationship between the idiopathic respiratory distress syndrome (IRDS) of prematurity and blood coagulation has been the subject of discussion for some time Particular attention has been paid to fibrinogen and fibrinolysis (1 10 11 15) The situation is complicated by the fact that in almost three quarters of the cases that come to autopsy cerebral haemorrhage usually in the region of a choroid vein is a major finding (4)

It would doubtless be pertinent to ascertain whether cerebral haemorrhage is of primary significance in these cases and possibly elicits symptoms which cannot be differentiated from those in genuine IRDS or even causes the full syndrome including the pulmonary changes or whether alternatively the hypoxia and hypercapnia of IRDS precipitate cerebral haemorrhage in these high risk infants (3) In the latter case discrimination between a loss of coagulation potential sequential to hepatic hypoxia (12) and intravascular coagulation (16) would be essential

In the present study immunological methods requiring very small blood samples were applied thus allowing serial assays of plasma fibrinogen and spontaneous in vitro fibrinolysis during the first 24 hours of life The examinations were carried out on normal premature infants and infants with IRDS of varying severity The results were analysed statistically [1]

MATERIALS AND METHODS

Plasma Samples

48 healthy premature infants with birth weights between 1100 g and 2500 g none of whom had signs of respiratory distress or died were assigned to the normal collective A second group comprised 19 premature infants with birth weights between 1190 g and 2280 g who presented the clinical signs of IRDS (high respiratory rate retractions grunting cyanosis, acidosis and a typical chest X ray) and recovered Finally fibrinogen and spontaneous fibrinolysis were determined in plasma from premature infants who died in the course of severe IRDS all of whom had hyaline membranes These were divided into two groups The first contained 11 infants with birth weights between 870 g and 2450 g who had no cerebral haemorrhage at autopsy the second 13 infants with massive cerebral haemorrhage at autopsy and 5 with sanguinolent liquor at postmortal puncture of the cisterna cerebellomedullaris (= 18 infants with birth weights between 1135 g and 2000 g) Six premature infants with birth weights between 1150 g and 1810 g dying of other causes such as oesophageal atresia septicaemia and cardiac malformations were neither assigned to these groups nor considered in the statistical analysis The plasma fibrinogen concentrations of these infants followed the typical rising course seen in normal infants during the first 24 hours of life (7)

The following four groups were compared by variance analysis and partly by multiple t tests

1 Normal premature infants
2 Premature infants with IRDS surviving
3 Premature infants dying of IRDS without cerebral haemorrhage
4 Premature infants dying of IRDS with cerebral haemorrhage

Table 1 shows the number of prematures in the various groups with weight ranges and mean values

The therapy of IRDS consisted of incubator care in high ambient oxygen concentrations and in the infusion of glucose solutions containing sodium bicarbonate for correction of acidosis according to serial

[1] Our thanks are due to Dipl Math Pfander Institut für Medizinische Statistik und Dokumentation der Universität Freiburg

8 Nilsson L R Soderhjelm L Hoglund N J & Tornblom N Infantilism hos spruepatient utan tarmbesvar *Nord Med 60* 1317 1958
9 Vinnik I E Kern F Jr & Struthers J E Jr Malabsorption and the diarrhea of diabetes mellitus *Gastroenterology 43* 210 1962
10 Visakorpi J K Immonen P & Kuitunen P Malabsorption syndrome in childhood The occurrence of absorption defects and their clinical significance *Acta Paediat Scand 56* 1 1967
11 Visakorpi J K & Immonen P Intolerance to cow s milk and wheat gluten in the primary malabsorption syndrome in infancy *Acta Paediat Scand 56* 49 1967

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Key words Coeliac disease malabsorption gluten free diet diabetic enteropathy a γ- A globulinaemia

Table 2 *Variance analysis for fibrinogen concentrations measured at different hours of age*

Age in hours		ms	df	F	Significance
1	Between groups	9041.28	3	3.37	
	Error	2679.28	18		
2	Between groups	1676.59	3	0.47	—
	Error	3554.18	19		
6	Between groups	34674.60	3	8.93	
	Error	3881.13	72		
12	Between groups	19039.15	3	4.07	
	Error	4676.71	53		
24	Between groups	10401.81	3	1.88	—
	Error	5539.46	65		

ms = mean square df = degrees of freedom

the fibrinogen determinations at various hours of life. As there were no differences between groups at the ages of 2 and 24 hours *t* tests were not carried out (see statistical methods). The fibrinogen values—mean and 95 % confidence limits—are shown in Fig. 1 for each group at each age. The statistical evaluations allow the following conclusions to be drawn.

1. No clear differences in plasma fibrinogen concentrations between normal premature infants (Group 1) and infants with IRDS who survived (Group 2) can be found in any of the age groups examined. The small difference at the age of one hour cannot be proved statistically.

Table 3 *Multiple t test for the fibrinogen concentrations measured at 1, 6 and 12 hours of age*

Age in hours	Group index	t value	Significance
1	1 2	2.06	—
	1 3	1.38	—
	1 4	3.02	
	2 3	0.08	—
	2 4	1.34	—
	3 4	0.88	—
	1 2 3 4	1.91	—
6	1 2	0.36	—
	1 3	1.08	—
	1 4	4.87	
	2 3	1.21	—
	2 4	4.36	
	3 4	2.31	
	1 2 3+4	3.73	
12	1 2	0.28	—
	1 3	0.43	—
	1 4	3.45	
	2 3	0.21	—
	2 4	2.91	
	3 4	2.24	
	1 2 3+4	2.40	

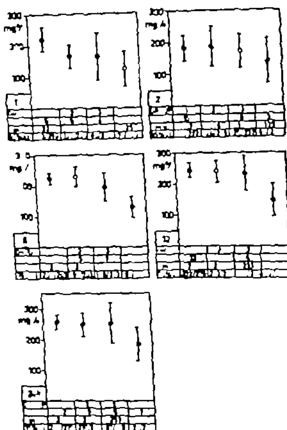


Fig. 1. Mean values and 95 % confidence limits for plasma fibrinogen concentrations of premature infants at 1, 2, 6, 12 and 24 hours of age. See text for definition of groups. *n* = number of infants examined, mv = mean value, 95 % cl = 95 % confidence limit.

Table 1 *Number of premature infants in the various groups with weight ranges and mean values*

Group	1	2	3	4
n	48	19	11	18
Birth weights				
Mean values	1900 g	1890 g	1480 g	1360 g
Ranges	1100–2500 g	1190–2280 g	870–2450 g	1135–2000 g

micro Astrup measurements Blood or plasma were not given in the cases examined

The samples consisted of 1 4 ml blood collected into 3 8 sodium citrate in the relationship 10 1 partly from venepuncture with conus free canula partly from indwelling umbilical venous catheters *No inhibitors of fibrinolysis were used* Sampling was carried out immediately after arrival of the infant on the premature ward and at the age of 6 12 and 24 hours After subsequent separation from the erythrocytes the plasma was frozen in all cases until the assay was undertaken

Antisera

Only monospecific antisera from rabbits were used which react solely with human fibrinogen and its split products (5)

Immunological assay of fibrinogen and fibrinolysis

Fibrinogen determinations were carried out by a modification of the radial immunodiffusion method (9) As fibrinogen split products can appear in the sera of normal premature infants (2) all samples were recalcified and split product determinations carried out on the sera The results of the fibrinogen measurements and those of the fibrinolysis assays were corrected accordingly

Fibrinolysis was determined by a new semi quantitative immunological method (6) All determinations were done in triplicate and the mean values used for the statistical analyses In our publication of 1968 the degree of fibrinolysis was expressed in terms of percentage of original fibrinogen As this definition is not suitable for statistical methods the degree of fibrinolysis is now defined as the amount (in mg/100 ml) of antiserum positive substance which remains in the serum after recalcification of the plasma and incubation at 37 C for 24 hours The expression in mg/100 ml is not quite correct as the substance set free from the clot during fibrinolysis has a lower molecular weight than fibrinogen (13) This difference is negligible however for the purpose of comparison between the four groups The unit mg is therefore retained for fibrinolysis

Statistics

Most of the infants were from 3 to 5 hours old at the time of admission so that these individuals first appear in the analysis at 6 hours of age As a consequence there are relatively few values for the first two hours of life Only 12 infants dying of IRS lived longer than 24 hours and a few measureme were marred by technical difficulties Venepunctu were occasionally impossible during shock and so of the plasma samples clotted spontaneously af thawing As it was therefore not possible to att values for all the infants at regular intervals separ statistical evaluations for each timepoint of sampl were necessary This means that for statistical p poses different groups were used for each sampl timepoint Comparison for error within groups therefore not strictly feasible

The values for a time sample (= age in hours) w examined by simple variance analysis using groups listed in the section Plasma samples If difference between the groups was significant (prob bility < 0 05) further comparison of groups by multiple *t* test ensued Not only every possible p but also Groups 1 and 2 (survivors) and 3 and (dead) were compared

In the tables showing variance analyses and *t* te the following key is used

- – not significant
- * *poor significance*
- ** significant
- * highly significant

Apparatus and instruments

The Eppendorf Microliter System comprising re tion tubes microburettes centrifuge and thermos was used throughout Spontaneous fibrinolysis of recalcified plasma samples was allowed to take pla in the polyethylene reaction tubes of this system 37 C in the thermostatic cell As the parts com into contact with the material under examination hydrophobic the system is particularly suitable for agulation studies with small quantities of plasm

The variance analyses were calculated on the IB 7040 computer in the University Dept of Mathem cs Freiburg

RESULTS

Fibrinogen

Table 2 shows the results of the variance anal ses Table 3 those of the multiple *t* tests f

Eppendorf Gerätebau D 2 Hamburg 63 Germar

1 Differences cannot be proved between the groups at 1 and 2 hours. Spontaneous fibrinolysis is equally pronounced and the values are higher than those found in adults (6).

2 At 6 hours significant differences can be found between the Groups 1 and 3 as well as 1 and 4. Fibrinolytic activity is significantly lower in all infants who died (Groups 3 and 4) as compared with normal infants and survivors from IRDS (Groups 1 and 2).

3 At 12 hours the results are comparable. Further differences are apparent between Groups 3 and 2 and 4 and 2. Those groups containing the infants who died (3 and 4) have highly significantly lower values for spontaneous fibrinolysis in vitro than those containing the survivors (1 and 2).

4 At 24 hours spontaneous clot lysis is hardly demonstrable for infants who died of IRDS whether they had cerebral haemorrhage (group 4) or not (group 3). Fibrin split products equivalent only to an average of 5 and 11 mg/100 ml plasma were found after incubation of the clot for 24 hours at 37 C. In these groups fibrinolysis is significantly weaker (Group 3) and poorly significantly weaker (Group 4) than in the control group (1). If all survivors are taken as a group and compared with those who died significantly *higher values* for spontaneous fibrinolysis are found in *in vitro* plasmas. Fibrinolytic activity is relatively uniform in the survivor groups (1 and 2) during the first 24 hours of life. This is not the case for those infants who died of IRDS. At the time of entry fibrinolytic activity is equal to that in the control group. Later a continuous fall in activity ensues during the observation period of 24 hours. This trend cannot be tested statistically however.

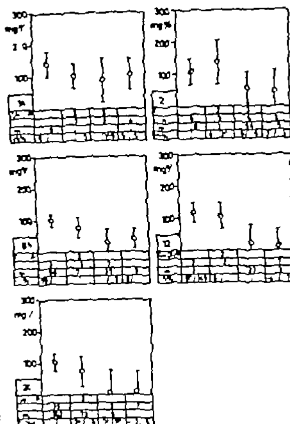


Fig. 2. Mean values and 95% confidence limits for *in vitro* plasma spontaneous fibrinolysis of premature infants at 1, 2, 6, 12 and 24 hours of age. See text for definition of groups. n = number of infants examined, mv = mean value, 95% cl = 95% confidence limit. (Negative values for confidence limits are not included in the diagrams.)

DISCUSSION

Fibrinogen

The results show that at no time during the first 24 hours of life *statistically* significant differences in plasma fibrinogen levels can be found between normal premature infants and such with the idiopathic respiratory distress syndrome who survive.

Only at the age of 1 hour is the mean value for survivors from IRDS (Group 2 = 168 mg/100 ml plasma) lower than for the control group (1 = 221 mg/100 ml). However this difference cannot be proved statistically on account of the large degree of variance.

Later the values for uncomplicated IRDS (Group 2) are partially even higher than the controls (Group 1). Uncomplicated IRDS is therefore not accompanied by hypofibrinogenaemia as assumed in the hypothesis of Stark *et al* (16). Postnatally no fall in plasma fibrinogen can be found in uncomplicated IRDS (Group 2) during the first 24 hours of life

Table 4 *Variance analysis for spontaneous fibrinolysis measured at different hours of age*

Age in hours		ms	df	F	Significance
1	Between groups	2101 22	3	0 98	—
	Error	2153 97	15		
2	Between groups	7464 12	3	2 36	—
	Error	3165 82	17		
6	Between groups	16891 19	3	5 18	**
	Error	3263 51	64		
12	Between groups	31253 29	3	6 23	***
	Error	5014 17	45		
24	Between groups	25970 53	3	3 72	
	Error	6977 78	56		

ms – *mean square df* – degrees of freedom

2 In all age groups only slightly lower fibrinogen concentrations were found in Group 3 (IRDS died no cerebral haemorrhage) as compared with Group 1 In no case was the difference of statistical significance

3 Only comparison of Group 4 (IRDS died cerebral haemorrhage) with the other three groups results in clear cut differences At the age of 1 and 12 hours the fibrinogen concentrations are significantly lower than those of the normal controls (Group 1 $p <$ 0 05) at the age of 6 hours this difference is highly significant ($p <$ 0 01) There are also differences between Groups 2 and 3 as compared with Group 4 some highly significant at the ages of 6 and 12 hours As more than half the infants in Group 4 died before attaining the 24th hour of life the differences found particularly as compared with Group 1 cannot be proved statistically

4 When the values for all of the survivors are compared as a group with the dead highly significant differences are found at 6 hours significant differences at 12 hours Here as in 1969 (7) a continuous rise in plasma fibrinogen level during the first 24 hours of life is apparent This rise took place in all four groups examined The trend is obvious but cannot be tested statistically as the populations were not identical at the time of sampling (see statistical methods) Particularly in Group 4 those prematures with low fibrinogen values tended to die before the 24th hour so that the relatively high overall 24 hours value could in par be due to the elimination of these low values

Fibrinolysis

Table 4 shows the results of the variance analyses Table 5 those for the *t* tests for fibrinolysis The mean values and 95° confid nce limits for the various age groups are shown in Fig 2 Negative values for confidence are no included Differences were found by varia ce analysis between the age groups 6 12 and 24 hours Only in these cases were *t* tests applied The results are as follows

Table 5 *Multiple t test for spontaneous fibrinolysis measured at 6 12 and 24 hours of age*

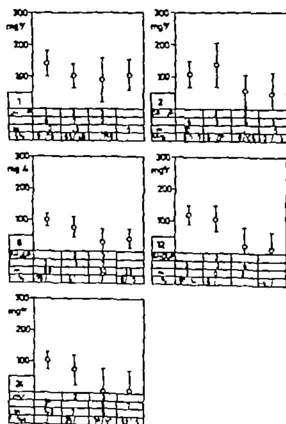
Age in hours	Group index		*t* value	Significance
6	1	2	1 13	—
	1	3	2 87	
	1	4	3 27	
	2	3	1 76	—
	2	4	1 73	—
	3	4	0 34	—
	1 + 2	3 4	3 29	*
12	1	2	0 44	—
	1	3	2 99	**
	1	4	3 51	
	2	3	2 41	
	2	4	2 84	*
	3	4	0 26	—
	1 + 2	3 + 4	4 12	*
24	1	2	1 07	—
	1	3	2 43	
	1	4	2 61	*
	2	3	1 55	—
	2	4	1 57	—
	3	4	0 14	—
	1 + 2	3 + 4	2 83	

1 Differences cannot be proved between the groups at 1 and 2 hours Spontaneous fibrinolysis is equally pronounced and the values are higher than those found in adults (6)

2 At 6 hours significant differences can be found between the Groups 1 and 3 as well as 1 and 4 Fibrinolytic activity is significantly lower in all infants who died (Groups 3 and 4) as compared with normal infants and survivors from IRDS (Groups 1 and 2)

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F g 2 Mean values and 95% confidence limits for in vitro plasma spontaneous fibrinolysis of premature infants at 1 2 6 12 and 24 hours of age See text for definition of groups n = number of infants examined mv mean value 95% cl = 95% confidence limit (Negative values for confidence limits are not included in the diagrams)

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Later the values for uncomplicated IRDS (Group 2) are partially even higher than the controls (Group 1) Uncomplicated *IRDS* is therefore not accompanied by hypofibrinogenaemia as assumed in the hypothesis of Stark *et al* (16) Postnatally no fall in plasma fibrinogen can be found in uncomplicated IRDS (Group 2) during the first 24 hours of life

On the contrary the mean value continues to rise during this period from 168 mg/100 ml to 248 mg/100 ml plasma Assuming fibrinogen consumption would entail assuming rapid fibrinogen production by the liver to compensate not only for the losses into the hyaline membranes of the lungs but also for the intravascular losses during hypothetical disseminated intravascular coagulation

The mean fibrinogen values in infants dying of IRDS without cerebral haemorrhage (Group 3) are lower than those in the control group in all assay periods In this group a rise in mean fibrinogen concentration during the first day of life is also apparent (Fig 1) All of these infants had hyaline membranes at autopsy This means that fibrinogen losses into the lungs are counteracted by increased fibrinogen synthesis in the liver Moreover other organs susceptible to fibrinogen deposition during intravascular coagulation were not examined histologically

The plasma fibrinogen concentrations of infants dying of IRDS with cerebral haemorrhage (Group 4) are already significantly lower than those in the control group at the age of one hour At 6 and at 12 hours of age the mean value for group 4 is significantly lower than all three remaining groups The difference at 24 hours could not be proved statistically because the low values in infants in group 4 dying earlier had been eliminated

As the half life of adult fibrinogen is three days the low values in group 4 cannot be explained purely by postnatal hypoxia with subsequent insufficiency in liver synthesis Loss of fibrinogen from the circulating plasma either during labour during the first hour of life or possibly even in utero must be assumed Complete cessation of fibrinogen production in the liver is highly improbable as the plasma concentration continues to increase in spite of postnatal loss into hyaline membranes This fact cannot be explained alone by release of fibrinogen from hypothetical depots Intravascular coagulation as a cause of hypofibrinogenaemia would have to have initiated and presumably also ceased, before the first hour of life

We have no explanation for these low fibrinogen values from the first hour of life onwards in infants dying of IRDS with cerebral haemorrhage as compared with those dying without cerebral haemorrhage Are the fibrinogen values in the former low enough to cause haemorrhage? The mean values of this group were never lower than 100 mg/100 ml plasma, that value considered critical in the adult Experimental work with newborn rabbits (8) and neuropathological findings (14) suggest that hypoxia can cause cerebral haemorrhage Possibly relative hypofibrinogenaemia in the hypoxic and hypercapnic infants of group 4 sufficed to precipitate cerebral haemorrhage

Fibrinolysis

The fibrinolytic activity in plasma of healthy premature infants and survivors from IRDS remains relatively constant during the first 24 hours of life Furthermore at no time could significant differences between normal controls (Group 1) and survivors from IRDS (Group 2) be found Spontaneous fibrinolysis in infants dying of IRDS (Groups 3 and 4) did not differ from that in the other two groups at the age of one hour Later however from the 6th hour of life onwards significantly lower values were found in infants dying than in survivors The fibrin clots from infants dying (Groups 3 and 4) were practically unlysable at 12 and 24 hours These findings of premortal loss of fibrinolytic activity compare well with those published by Markarian *et al* (10) who used the euglobulin lysis method

This fall in fibrinolytic activity *in vitro* in Groups 3 and 4 could be caused by consumption or inactivation of fibrinolytic potential As the lower plasminogen concentrations found in these groups do not fall further during the first 24 hours of life as preliminary results show alterations of plasminogen activator systems or the influence of antiplasmin rather than a loss of plasminogen must be assumed

Quantitative analysis of fibrin and fibrino

gen split products in fresh serum did not elucidate the state of the fibrinolytic system *in vivo*. Split products were found in concentrations surpassing 8 mg/100 ml in the sera of 2 infants from Group 4, 2 infants from Group 3 and 1 infant from Group 2, but also in the sera of 8 healthy premature infants. Thus no correlation between the severity of IRDS and presence of fibrin and fibrinogen split products could be construed. Bonifaci *et al.* (2) also found split products in cord blood of healthy newborn infants. The estimation of split products therefore has no prognostic value.

SUMMARY

Serial assays of plasma fibrinogen and fibrinolysis in normal premature infants and premature infants with the Idiopathic Respiratory Distress Syndrome (IRDS) were made during the first day of life.

During the first 24 hours of life there were no significant differences in fibrinogen concentration between infants with uncomplicated IRDS and normal infants. In those infants who died of IRDS without cerebral haemorrhage slightly lower fibrinogen values were found. Premature infants who died of IRDS with cerebral haemorrhage had lower fibrinogen values than those in the other three groups from the first hour of life onwards. The plasma fibrinogen concentration rose during the first 24 hours of life in all four groups. The degree of spontaneous fibrinolysis *in vitro* was equal in all four groups at the second and sixth hour of life. Fibrinolysis subsequently fell to hardly measurable values in infants who died whereas it remained relatively constant in normal premature infants and survivors from IRDS.

REFERENCES

1 Ambrus C M, Weintraub D H, Dunphy D, Dowd J E, Niswander K R & Ambrus J L. The fibrinolysin system in pathogenesis and therapy. *Pediatrics 32* 10 1963
2 Bonifaci E, Raggio P & Gravina E. Demonstration of split products of fibrinogen in the blood of normal newborn. *Biol Neonat 12* 23 1968
3 Harrison V C, Heese H & Klein M. Intracranial haemorrhage associated with hyaline membrane disease. *Archiv Dis Child 43* 116 1968
4 Kabus K, Schenck W & Künzer W. Atemnotsyndrom und Blutgerinnungsaktivität. *Z Kinderheilk 105* 55 1969
5 Karitzky D & Künzer W. Herstellung eines monospezifischen Antiserums gegen menschliches Fibrinogen. *Thrombos Diathes Haemorrh 18* 532 1967
6 — Eine semiquantitative immunologische Methode zur Fibrinolysebestimmung. *Thrombos Diathes Haemorrh 19* 533 1968
7 Karitzky D, Pringsheim W, Zimmer O & Künzer W. Veränderungen der Fibrinogenkonzentration im Plasma gesunder Frühgeborener am ersten Lebenstag. *Z Kinderheilk 105* 73 1969
8 Ludwig H. Mikrozirkulationsstörungen und Diapedeseblutungen im fetalen Gehirn bei Hypoxie. *Bibliotheca Gynecologica* (Basel) 46 1968
9 Mancini G, Carbonara A O & Heremans J F. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry 2* 235 1965
10 Markarian M, Lindley A, Lubchenco L O & Githens J H. Fibrinolytic activity in premature infants with the respiratory distress syndrome. *J Pediat 67* 957 1965
11 Markarian M, Githens J H, Jackson J J, Bannon A E, Lindley A, Rosenblut E, Martorell R & Lubchenco L O. Fibrinolytic activity in premature infants. *Amer J Dis Child 113* 312 1967
12 Markarian M, Lindley A, Jackson J J & Bannon A. Coagulation factors in pregnant women and premature infants with and without the respiratory distress syndrome. *Thrombos Diathes Haemorrh 17* 585 1967
13 Nussenzweig V, Seligmann M & Grabar P. Les produits de dégradation du fibrinogène humain par la plasmine. *Ann Inst Pasteur 100* 490 1961
14 Towbin A. Cerebral intraventricular hemorrhage and subependymal matrix infarction in the fetus and premature newborn. *Amer J Path 52* 121 1968
15 Samartzis E A, Cook C D & Rudolph A J. Fibrinolytic activity in the serum of infants with and without the hyaline membrane syndrome. *Acta Paediat Scand 49* 727 1960
16 Stark C R, Abramson D & Erkan V. Intravascular coagulation and hyaline membrane disease of the newborn. *Lancet 1* 1180 1968

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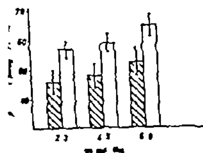


Fig 1 Average values and range of phagocytosis of *E coli* in various ages Children with rickets in striped columns controls in white columns

lined by Kabat & Mayer (7) was used to determine the 50% hemolytic units of complement activity (C H units)

Bactericidal activity Bactericidal activity of leukocytes was evaluated by the method described by Miller & Buckley (13)

Immunoglobulin levels The evaluation of IgG and IgM levels was performed by means of the quantitative immunoprecipitation method on Partigen plate (Behringwerke AG Marburg) according to Becker *et al* (1)

Statistical method The variables obtained were subjected to the *t* test and the probability was calculated

RESULTS

Dependence of opsonins on the age and Ca × P *index* Following the dependence of heat stable serum opsonizing activity on the age the increase of their values with growth can be seen (Fig 1) There are significant differences between rickets and controls ($p < 0.005$) In addition the lowering of heat stable opsonin levels is connected with the severity of the illness expressed by Ca × P index (Fig 2)

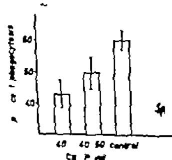


Fig 2 Average values and range of phagocytosis of *E coli* in groups with different Ca P indexes

Table 1 *Phagocytosis of E coli in normal serum and serum in rickets by normal and rickets leukocytes*

Source of serum	Source of leukocytes	
	Normal	Rickets
Normal	60 %	63
Rickets	42 "	46 "

Bactericidal activity The patient s leukocytes were equally as effective in killing of *E coli* as were the leukocytes from normal controls Mean number of viable bacteria after 1 hour has been 2×10^4 in normal children and 5×10^4 in rickets after 2 hours 1.5×10^3 and 4×10^3 respectively No significant differences were noted either in the rate of killing or in the number of viable bacteria at the end of the test period

Leukocytes and serum in rickets Serum and peripheral leukocytes from normal subjects and children with rickets were compared in the phagocytic tests which were performed in 5 cases (Table 1) As seen the serum was responsible for the variation in phagocytosis

Serum opsonizing activity The two groups of 7 children each were tested for serum opsonizing activity for *E coli* The mean phagocytosis in unheated serum was 56 per cent for children with rickets and 74 per cent for controls Heated aliquots of the same serums resulted in phagocytosis of 41 and 58 per cent respectively Thus the heat stable component of opsonin seems to be essential for diminished phagocytosis in rickets (Fig 3)

Complement activity Since it has been demonstrated that hemolytic complement may take part in the process of opsonization complement activity was compared in the serums of the normal controls and those of children with rickets The 50 per cent hemolytic units of complement are seen to be similar in these two groups (Fig 3)

Test for serum phagocytosis inhibitor In view of marked depression of phagocytosis in rickets an inhibitor was considered and tested

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EVALUATION OF PHAGOCYTOSIS IN RICKETS

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Vitamin D deficient rickets is conspicuously accompanied by infections (8) Here the problem arises, of whether these accompanying diseases are caused by an increased exposure to infection or by a certain insufficiency of the defense system of an organism

Cases of rickets occur frequently in families of lower social level where there is an increased possibility of food contamination or inhalation infection On the other hand however a certain damage to the defense system of the organism evoked by rickets may also cause the increased incidence of infections This possibility is supported by the observation of a decreased cellular and humoral immunity in young rats with experimentally induced rickets (18) In an attempt to study this problem in children we carried out examinations of phagocytosis bacteriotoxicity and of complement levels in infants with rickets *Escherichia coli* was used in all experiments because children are usually exposed to this antigen in the early stages of life and subsequently produce antibodies against this bacteria (9 10)

MATERIAL AND METHODS

Studies were performed on 30 children with rickets verified by x ray examination biochemical findings and craniotabes (6) (13 boys 17 girls) and on 33 healthy children (15 boys 18 girls) The children were divided into 3 age groups Rickets groups 2–3 months (9 cases) 4–5 months (14 cases) 6–8 months (7 cases) control groups 2–3 months (12 cases) 4–5 months (12 cases) 6–8 months (9 cases) The determinations of calcium and phosphorus blood levels in all cases were performed and Ca × P indexes calculated (4) There were 9 children with Ca × P index below 40 and 12 infants with this index between 40 and 50 in the rickets group

Blood The blood was obtained by venipuncture and collected in test tubes Aliquots of 3 ml were placed in equal volumes of 6 per cent clinical dextran molecular weight 75 000 (Dr Fresenius Bad Homburg) in physiologic saline solution with 70 m heparin 135 000 I U /g (Werner Schur Hamburg) per 100 ml

Preparation of leukocytes Aliquots of blood in dextran solution as described above were sedimented 1 hour at room temperature The leukocyte rich supernatant was removed and washed three times in TC medium 199 with Earlés BSS (Serva Heidelberg) and 0 5 human serum albumin (Behringwerke AG Marburg) Amounts of $1\ 25 \times 10^{6}$ leukocytes per ml were used within an hour of preparation

Preparation of serum Blood was drawn by venipuncture and allowed to clot at room temperature Serums obtained were either heated at 56 C in a water bath for 30 min or used fresh without any additional treatment

Preparation of bacteria Bacterial suspensions were made from 24 hours old cultures of *Escherichia coli* B 2340 grown in CT medium (Bactotrypton 10 g Yeast extract 1 g NaCl 8 g ad 1000 ml H_2O) with 1 mM of calcium chlorid and 1 of glucose The suspensions were washed twice with isotonic saline and resuspended after counting in Helber chamber so that 0 025 ml contained 25 10 bacteria

Phagocytic test The experimental procedure used in studying the phagocytic rate was essentially that of Winkelstein & Drachman (21) Aliquots of leukocyte suspension containing $12\ 5 \times 10$ cells per ml were placed in pyrex tubes After centrifugation at 900 rpm for five minutes the supernatant was removed Bacteria in the amount of 25×10 in 0 025 ml were added as well as 0 5 ml of either heated or unheated serum diluted 1 10 in TC medium 199 After gentle mixing the tubes were sealed for 30 min and then stained with methylene blue The percentage of phagocytosis was determined by counting of 300 leukocytes and scoring of those with ingested bacteria

Hemolytic assay of complement The method out

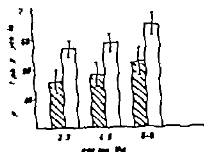


Fig 1 Average values and range of phagocytosis of *E coli* at various ages Children with rickets in striped columns controls in white columns

lined by Kabat & Mayer (7) was used to determine the 50% hemolytic units of complement activity (C' H units)

Bactericidal activity Bactericidal activity of leukocytes was evaluated by the method described by Miller & Buckler (13)

Immunoglobulin levels The evaluation of IgG and IgM levels was performed by means of the quantitative immunoprecipitation method on Partigen plate (Behringwerke AG Marburg) according to Becker *et al* (1)

Statistical method The variables obtained were subjected to the t test and the probability was calculated

RESULTS

Dependence of opsonins on the age and Ca × P index Following the dependence of heat stable serum opsonizing activity on the age the increase of their values with growth can be seen (Fig 1) There are significant differences between rickets and controls ($p < 0.005$) In addition the lowering of heat stable opsonin levels is connected with the severity of the illness expressed by Ca × P index (Fig 2)

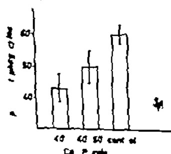


Fig 2 Average values and range of phagocytosis of E coli in groups with different Ca × P indexes

Table 1 *Phagocytosis of E coli in normal serum and serum in rickets by normal and rickets leukocytes*

Source of serum	Source of leukocytes	
	Normal	Rickets
Normal	60%	63%
Rickets	42%	46%

Bactericidal activity The patient's leukocytes were equally as effective in killing of *E coli* as were the leukocytes from normal controls Mean number of viable bacteria after 1 hour has been 2×10^4 in normal children and 5×10^4 in rickets after 2 hours 1.5×10^3 and 4×10^3 respectively No significant differences were noted either in the rate of killing or in the number of viable bacteria at the end of the test period

Leukocytes and serum in rickets Serum and peripheral leukocytes from normal subjects and children with rickets were compared in the phagocytic tests which were performed in 5 cases (Table 1) As seen the serum was responsible for the variation in phagocytosis

Serum opsonizing activity The two groups of 7 children each were tested for serum opsonizing activity for *E coli* The mean phagocytosis in unheated serum was 56 per cent for children with rickets and 74 per cent for controls Heated aliquots of the same serums resulted in phagocytosis of 41 and 58 per cent respectively Thus the heat stable component of opsonin seems to be essential for diminished phagocytosis in rickets (Fig 3)

Complement activity Since it has been demonstrated that hemolytic complement may take part in the process of opsonization complement activity was compared in the serums of the normal controls and those of children with rickets The 50 per cent hemolytic units of complement are seen to be similar in these two groups (Fig 3)

Test for serum phagocytosis inhibitor In view of marked depression of phagocytosis in rickets an inhibitor was considered and tested

in 5 cases with Ca × P index below 40 If some factor in the test serum had interfered with phagocytosis serum added to normal serum should have depressed phagocytosis As seen in Table 2, this was not the case

Immunoglobulin levels The average values of IgG in three age groups (2–3 4–5 6–8 months) were 520 mg 430 mg and 510 mg/100 ml serum by rickets and 480 mg 410 mg and 540 mg/100 ml serum by controls the levels of IgM 42 mg 49 mg and 51 mg/100 ml serum by rickets and 40 mg 53 mg and 50 mg/100 ml serum by controls No significant differences were noted between these two groups

DISCUSSION

Rickets occurs during the suckling period at a time when immunological reactions against many antigens are not yet developed (15) When looking for the origin of decreased serum opsonizing activity for *Escherichia coli* and interpreting the results in relation to the age, it is necessary to realize that normally the *development of an intestinal flora is essential* to provoke antibody formation against this bacteria (17) The infant begins to produce his own antibodies against *E coli* during the 2nd to 4th month of life (10) Because antibodies against gram negative bacteria which take part in serum opsonizing activity are predominantly gamma M globulins (14) the role

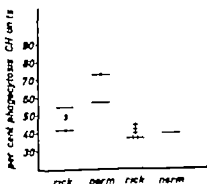


Fig 3 Phagocytosis of *E coli* in various serums and complement levels Tests in heated serums are indicated by solid dots and those in unheated serums by open circles Complement levels are indicated by crosses

Table 2 *Phagocytosis of E coli in various combinations of normal serum and serum from patients with rickets*

Serum			
Rickets	Normal	Buffer	of phag
—	0 1 ml	0 4 ml	64
0 4 ml	0 1 ml	—	61
0 4 ml	—	0 1 ml	51
0 1 ml	—	0 4 ml	38

of diaplacentar transport is minimal These antibodies are supplied in various mammals such as the cow and pig via colostrum but there is no appreciable absorption by the suckling human baby (9) The 0 antigen of *E coli* is necessary for the production of opsonins the titres of which rise during the first year of life (11) For the opsonisation of a smooth strain of *E coli* which is used in this study the pres*ence of a specific antibody is important* (12) In spite of the existence of many intestinal *E coli* strains during suckling period which differ in every individual (20) the dependence of serum opsonizing activity for *E coli* B 2340 was *observed with respect to the heat stable* component

Due to the clinical symptoms of rickets varying in intensity the varying ages of the tested patients and since there are no signs of rickets among *the healthy children the phagocytosis* values must be related to same constant Therefore in agreement with Fanconi (4) the Ca × P index was applied As our results indicate rickets is accompanied by decreased phagocytosis which is *dependent on* age and Ca × P index This is due to a lower level of the heat stable component of opsonin—i e antibody The fact that the heat labile component is the same as in healthy children is verified by parallel determination of *complement*, which is supposedly the main component of the heat labile opsonin (16) The reason for decreased opsonisation is not the decrease of total immunoglobulin levels no differences were found in total serum *IgG and IgM against* controls

The levels of agglutinins for *E coli* in the first months of life are either very low or zero

(19) Based upon "in vivo" clearance studies with *E coli* it was shown however that the opsonizing ability of the immune serum was a more sensitive measure of antibody activity than agglutination (2) The cause of the decreased serum opsonizing activity in rickets may be a certain impairment of the specific component of immunity response of the organism Because of the factors which influence quantity and composition of the intestinal bacteria of infants (3) there is no reason to expect differences in antigenical stimulation between normal children and those with rickets It has been proven that the concentration of calcium influences phagocytosis in vitro (5) However this fact can not play a role in the case of rickets because of very small differences of blood calcium levels in comparison with normal children Also according to our results the bactericidal capacity in rickets does not change Leukocytes are functionally fully competent, and additionally there was no evidence for the existence of a serum inhibitor of phagocytosis Thus the alterations of phagocytosis in rickets seem to be predominantly the consequence of an impaired antibody production

SUMMARY

Children with rickets have been shown to have the normal bactericidal capacity of leukocytes for *Escherichia coli* but impaired phagocytosis of this bacteria It has been proven that the heat stable component of opsonin is responsible for this fact Dependence of serum opsonizing activity on age and Ca × P index in rickets have been observed The findings provide a possible explanation for clinical observations on diminished resistance to infection in rickets

ACKNOWLEDGEMENTS

We are indebted to Prof E. Wecker for helpful criticism and supply of bacterial strains Prof E Golmer for advice and help in statistical matters and Dr H Neumayer for referring 2 patients We are also indebted to Prof H Schelp for his help in English correction

REFERENCES

1 Becker W Rapp W, Schwick H G & Storiko K Methoden zur quantitativen Bestimmung von Plasmaproteinen durch Immunpräzipitation *Z Klin Chem* 6 113 1968

2 Benacerraf B Sebestyen M M & Schlossman S A quantitative study of the kinetics of blood clearance of P[32] labelled *E coli* and Staphylococci by the reticuloendothelial system *J Exp Med 110* 27 1959

3 Braun O H Ernährung und Darmflora im Säuglingsalter *Medizin und Ernährung* 3 83 1962

4 Fanconi G & Wallgren A *Lehrbuch der Pädiatrie* Schwabe & Co Verlag Basel and Stuttgart 1967 8th edition p 171

5 Henon M & Delaunay A Action exercée in vitro par les ions Ca et Mg sur la sensibilité chimiotactique et le pouvoir phagocytaire de leucocytes polynucléaires *Ann Inst Pasteur 111* 85 1966

6 Hovels O Bock H Rasche F & Sommerburg O Über die Häufigkeit weiterer rachitischer Zeichen bei Kraniotabes *Z Kinderheilk* 69 361 1951

7 Kabat E & Mayer M M *Experimental immunochemistry* Thomas Springfield Illinois *1961 p 149*

8 Khamitova T M The immunoreactive condition of children suffering from rickets *Pediatr Akus Ginek* (Kiev) 6 26 1967

9 Kunin C M Antibody distribution against non enteropathic E coli *Arch Intern Med* 110 676 1962

10 Lodinová R Jouja V & Lanc A Influence of the intestinal flora on the development of immune reactions in infants *J Bact 93* 797 1967

11 Michael J G & Rosen F S Association of natural antibodies to gram negative bacteria with the γ macroglobulins *J Exp Med 118* 619 1963

12 Miler I Tlaskalova, H Mandel L & Travnicek J The phagocytic activity of reticuloendothelial system of newborn precolostral pigs to smooth and rough *E coli Folia Microbiol 13* 472 1968

13 Miller M E & Buckler J M Intracellular survival and growth of Serratia marcescens following phagocytosis by human leukocytes *J Infect Dis* 118 149 1968

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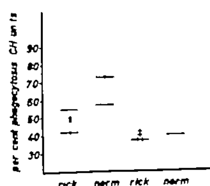


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Due to the clinical symptoms of rickets varying in intensity the varying ages of the tested patients and since there are no signs of rickets among the healthy children the phagocytosis values must be related to same constant Therefore in agreement with Fanconi (4) the Ca × P index was applied As our results indicate, rickets is accompanied by decreased phagocytosis which is dependent on age and Ca × P index This is due to a lower level of the heat stable component of opsonin—i e antibody The fact that the heat labile component is the same as in healthy children is verified by parallel determination of complement which is supposedly the main component of the heat labile opsonin (16) The reason for decreased opsonisation is not the decrease of total immunoglobulin levels no differences were found in total serum IgG and IgM against controls

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SUMMARY

Children with rickets have been shown to have the normal bactericidal capacity of leukocytes for *Escherichia coli* but impaired phagocytosis of this bacteria. It has been proven that the heat stable component of opsonin is responsible for this fact. Dependence of serum opsonizing activity on age and Ca × P index in rickets have been observed. The findings provide a possible explanation for clinical observations on diminished resistence to infection in rickets.

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REFERENCES

1. Becker, W., Rapp, W., Schwick, H. G. & Storiko, K.: Methoden zur quantitativen Bestimmung von Plasmaproteinen durch Immunpräzipitation. *Z. Klin. Chem.* 6: 113, 1968.
2. Benacerraf, B., Sebestyen, M. M. & Schlossman, S.: A quantitative study of the kinetics of blood clearance of P³² labelled *E. coli* and Staphylococci by the reticuloendothelial system. *J. Exp. Med.* 110: 27, 1959.
3. Braun, O. H.: Ernährung und Darmflora im Säuglingsalter. *Med. u. Ernährung* 3: 83, 1962.
4. Fanconi, G. & Wallgren, A.: *Lehrbuch der Pädiatrie.* Schwabe & Co. Verlag, Basel and Stuttgart 1967, 8th edition, p. 171.
5. Henon, M. & Delaunay, A.: Action exercée in vitro par les ions Ca⁺⁺ et Mg⁺⁺ sur la sensibilité chimiotactique et le pouvoir phagocytaire de leucocytes polynucléaires. *Ann. Inst. Pasteur* 111: 85, 1966.
6. Hovels, O., Bock, H., Rasche, F. & Sommerhage, O.: Über die Häufigkeit weiterer rachitischer Zeichen bei Kraniotabes. *Z. Kinderheilk.* 69: 361, 1951.
7. Kabat, E. & Mayer, M. M.: *Experimental immunochemistry.* Thomas, Springfield, Illinois 1961, p. 149.
8. Khaustova, T. M.: The immunoreactive condition of children suffering from rickets. *Pediatr. Akus. Ginek.* (Kiev) 6: 26, 1967.
9. Kunin, C. M.: Antibody distribution against non enteropathic *E. coli.* *Arch. Intern. Med.* 110: 676, 1962.
10. Lodinova, R., Jouja, V. & Lanc, A.: Influence of the intestinal flora on the development of immune reactions in infants. *J. Bact.* 93: 797, 1967.
11. Michael, J. G. & Rosen, F. S.: Association of natural antibodies to gram negative bacteria with the γ macroglobulins. *J. Exp. Med.* 118: 619, 1963.
12. Miler, I., Tleskalova, H., Mandel, L. & Travnicek, J.: The phagocytic activity of reticuloendothelial system of newborn precolostral pigs to smooth and rough *E. coli.* *Folia Microbiol.* 13: 472, 1968.
13. Miller, M. E. & Buckler, J. M.: Intracellular survival and growth of Serratia marcescens following phagocytosis by human leukocytes. *J. Infect. Dis.* 118: 149, 1968.
14. Robbins, J. B., Kenny, K. & Suter, E.: The isolation and biological activities of rabbit gamma M and gamma G anti-Salmonella typhimurium antibodies. *J. Exp. Med.* 122: 385, 1965.
15. Smith, R. T. & Eitzman, D. V.: Characterization of the response of the human infant and adult to immunization with Salmonella vaccines. *Pediatrics* 33: 163, 1964.
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17 Stetson C A *Endotoxins and bacterial allergy Cellular and humoral aspects of the hypersensitive state* Hoeber medical division Harper & Row Publishers New York 1959
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Key words Rickets phagocytosis opsonisation

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FINE NEEDLE ASPIRATION BIOPSY OF HUMAN LIVER FOR ENZYMATIC DIAGNOSIS OF GLYCOGEN STORAGE DISEASE AND GARGOYLISM

A LUNDQUIST and P A OCKERMAN

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As has already been discussed in an earlier paper (10) there is a great need for safe and accurate methods for the diagnosis of glycogen storage disease The advent of diagnostic assays on blood cells has not made unnecessary analyses on liver biopsy specimens in many cases In order to be able to use a biopsy technique involving less risk for the patients than those hitherto used the fine needle aspiration biopsy technique as described by Soderstrom (12) was introduced Methods were worked out (10) allowing the analyses for the diagnosis of glycogen storage disease to be made on the small fragments of liver tissue obtained

In earlier papers (5 6) normal values were given of several enzymes of glycogen metabolism and glucose production as well as enzymes localized intracellularly to the lysosomes This communication presents the application of the technique in the diagnosis of glycogen storage disease and gargoylism of the Hurler type (mucopolysaccharidosis type I according to McKusick (7)) In this latter disease a deficiency of the activity of β galactosidase has recently been demonstrated (11)

MATERIAL AND METHODS

Biopsy technique and enzyme assay

As in ref 5 and 6 the patient was fasted over night In the morning a fine needle aspiration biopsy was performed once or twice in local anaesthesia The procedure for biopsy and immediate preparation of the specimen obtained was as described in an earlier communication (5)

One homogenate in sucrose EDTA was used for analyses of glucose 6 phosphatase phosphorylase amylo-1 6-glucosidase and protein as described earlier (10) and a second homogenate in water for assay of glycogen α glucosidase (10) β galactosidase β glucuronidase N acetyl β glucosaminidase (11) and protein (4) The fact that the fragments were not weighed minimized the risk for inactivation of labile enzymes Accurate weighing would also have been impossible since washing from blood was necessary Sucrose EDTA was necessary to stabilize glucose 6-phosphatase (5) water to activate all lysosomal enzymes (6)

Patients

All patients with glycogen storage disease had symptoms and signs in accordance with a diagnosis of hepatomegalic glycogenosis Glycogen and enzyme assays were performed with earlier described methods (2 3 9) on liver (cases 1 3 and 4) and muscle biopsies (cases 1 and 2) and allowed a definite diagnosis to be made of glycogenosis type I in case 4 (a sister has been reported as case 7 in ref 9) type III in case 2 and type VI in cases 1 and 3

The patients with gargoylism all had symptoms and signs in accordance with a diagnosis of Hurler's syndrome and excreted large amounts of glycosaminoglycans (13) mainly dermatan sulfate and to a less extent heparan sulfate (8) in their urines

RESULTS

Glycogen storage disease

Fragments of liver tissue from case 1 obtained by fine needle aspiration biopsy were used for

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DISCUSSION

The results given for comparison represent to our knowledge the only values published for strictly normal healthy individuals However all controls were adults and may therefore not represent an optimal reference In spite of its relative freedom from risks to the patients fine needle aspiration biopsies were not considered feasible in normal children Also it is to be expected that unspecific changes in the form of increased or decreased values for some enzyme activities will be obtained as a result of the disease Consequently only very clear differences can be easily interpreted We consider that such clear differences existed in the seven patients described in this communication

Since the first enzyme defect in glycogen storage disease was described in 1952 (1) a development of the methods of enzyme assays has occurred The amount of tissue needed has diminished Thus it was considered a great step forward when Hers in 1959 (2) published methods allowing the assay of glycogen glucose 6-phosphatase amylo-1 6-glucosidase and phosphorylase to be made on only about 10–20 mg of tissue To be able to analyse α glucosidase a further 5–10 mg of liver was needed (3) The present methods allowing analyses on 0 5–2 mg thus represents a sensitivity at least 10 times higher

The main advantage of the suggested procedure is that it is less risky to the patient than other liver biopsy procedures The following draw backs can be mentioned 1) The assays are slightly more time-consuming than Hers methods (2 3) 2) It has not been proved that samples can be sent from one hospital to another 3) No liver tissue can be saved frozen for future use since all the material obtained is used for the analyses

Nevertheless the suggested procedure would represent a recommendable technique in cases of metabolic diseases in which analyses of more easily obtainable tissue such as blood cells are not sufficient or when general anaesthesia or larger diameter needle biopsy is considered dangerous

SUMMARY

Using a needle outer diameter 0 7 mm liver biopsies were performed in local anaesthesia The small fragments of tissues obtained 0 5–2 mg were used for assay of enzymes and glycogen in one case each of glycogenosis type I and II and two of type VI and three cases of Hurler's syndrome The enzyme defects known to exist in liver in these diseases could be demonstrated while no defects were found of related enzymes It is suggested that fine needle aspiration biopsy of the liver should be used for diagnostic purpose with assay of relevant enzymes in glycogen storage disease and gargoylism and maybe also other metabolic diseases This method involves less risk for the patient than conventional liver biopsy procedures and gives information that in many cases cannot be obtained by analyses on other tissues

ACKNOWLEDGEMENT

The skilful technical assistance of Mrs C Lindfors Miss S Olsson and Miss M Sjoland is gratefully acknowledged This investigation was supported by the Swedish Medical Research Council (Grants K67 19X 2222-01 and B68 13X 2307-01) The University of Lund and Forssmansbloommans Riksforbund

REFERENCES

1 Cori G T Glycogen Structure and enzyme deficiencies in glycogen storage disease *Harvey Lectures 48* 145 1952 53
2 Hers H G Études enzymatiques sur fragments hépatiques Application à la classification des glycogénoses *Rev Internat Hépatol 9* 35 1959
3 — α glucosidase deficiency in generalized glycogen storage disease (Pompe's disease) *Biochem J 86* 11 1963
4 Lowry O H Rosebrough N J Farr A L & Randall R J Protein measurement with the Folin phenol reagent *J Biol Chem 193* 265 1951
5 Lundquist, A Öckerman P A & Schersten B Fine needle aspiration biopsy of the liver in healthy adults Activity of enzymes of glycogen utilization and glucose production. *Enzym Biol Clin 10* 8 1969
6 Lundquist A & Öckerman P A Fine needle aspiration biopsy of the liver in healthy adults Activity of lysosomal acid hydrolases. *Enzym Biol Clin 10* 300 1969

Table 1 *Enzyme activities and glycogen concentration in human liver*

Enzyme activities expressed as μmoles substrate split/g protein/min except for amylo 1 6-glucosidase given as μmoles product formed Glycogen concentration given as g/g protein

		Glucose 6 phosphatase	Phosphorylase	Amylo 1 6 glucosidase	α glucosidase	Glycoge
Controls[a] n = 14–16	Mean	41 6	128 7	4 20	*14 2*	*0 206*[b]
	Range	(23 5–73 8)	(72 8–230 3)	(2 08–7 49)	(2 49–34 6)	(0 096–0
Glycogen storage disease						
Case 1 male 5		15 9	36 6	1 60	1 30	0 609
Case 2 male 1		*8 4*	280 0	*0*	*10 4*	2 34
Case 3 female 1		36 7	36 2	2 97	—	2 12
Case 4 female 1		0 0	160 0	2 92	4 71	1 28

[a] Healthy adults (5)
[b] Biopsy taken at operation (10)

analyses of enzyme patterns and glycogen as shown in Table 1 It is evident that the glycogen concentration was increased The activity of glucose 6-phosphatase amylo-1 6-glucosidase and α glucosidase was only slightly decreased a very definite activity still remaining This excludes a diagnosis of glycogenosis type I II and III Phosphorylase activity was decreased to about 20 of the mean activity in the controls This is in good accordance with a diagnosis of glycogenosis type VI where usually one finds increased glycogen and a decrease of the activity of phosphorylase to 1/2 to 1/4 of the normal It is concluded that a correct diagnosis could be made on the results of the analyses on the fine needle biopsy specimen

In case 2 a very high level of glycogen was found Glucose 6 phosphatase activity was slightly decreased a very definite activity maining α glucosidase and phosphorylase tivities were high No activity of amylo glucosidase was found The results are in cordance with a diagnosis of glycogen sto disease type III In case 3 a similar reaso as in case 1 can be applied and a diagn be made of glycogen storage disease type

The findings in case 4 very clearly ar accordance with a diagnosis of glycogen s age disease type I

Gargoylism

Table 2 shows the result in the patients gargoylism Clearly a deficiency of β gala sidase could be diagnosed from these res while the activities of β glucuronidase and acetyl β-glucosaminidase were not decrease

Table 2 *Activity of acid hydrolases in fine needle biopsies of human liver*

All values expressed as μmoles substrate split/g protein/min

		β galactosidase	β glucuronidase	N acetyl β glucosaminidase
Controls[a] (n = 24)	Mean (Range)	4 79 (1 69–11 7)	4 32 (2 15–9 16)	13 3 (7 59–29 9)
Gargoylism				
Case 1 female 8		0 27	1 98	13 5
Case 2 female 5		0 48	9 25	75 6
Case 3 female 8		0 88	14 1	90 2

[a] Healthy adults (6)

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CYTOMEGALOVIRUS INFECTIONS IN DIFFERENT GROUPS OF PAEDIATRIC PATIENTS

GUN CARLSTRÖM and BIRGITTA JALLING

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According to previously presented findings cytomegalovirus (CMV) infections frequently occur in the Swedish population (2) The high proportion of individuals found to carry antibodies against CMV corresponds well with findings from other geographical areas (10 16 19) The introduction of viral diagnostic procedures for CMV infections has been followed by a gradual delineation of the clinical picture of both congenital (5 9 12 14 18 21) and acquired (1 3 4 5 7 8 10 11 12 13 17 18 20) CMV infections However many problems await further elucidation The role of CMV as a pathogen in different clinical syndromes has not yet been completely explored nor has the incidence of inapparent CMV infections and minor clinical illnesses with subtle or unspecific manifestations been thoroughly investigated Because of observations of the persistence of infection and the reactivation of dormant virus it is difficult to assess both in acute and chronic cases whether the disease is caused by a primary infection or a reactivation Furthermore the mode of transmission of infection is yet not fully understood

The present investigation was undertaken in an attempt to elucidate some of these problems Sero-epidemiological and virus isolation studies were carried out at Karolinska sjukhuset from 1963 through 1967 in different groups of children

MATERIAL AND METHODS

Clinical material Seven groups of subjects were studied

Group 1 Newborn infants with neonatal jaundice but without blood group incompatibility and without other signs of illness

Group 2 Infants up to six months of age with suspected infectious disease and CMV infection questioned

Group 3 Children seven months to fifteen years of age admitted with various acute infectious diseases

Group 4 Children seven months to five years of age admitted to the Ear Nose and Throat Department with acute subglottic laryngitis

Group 5 Seven months to fifteen years old children with congenital or acquired disease of the central nervous system admitted for neurology study

Group 6 Seven months to fifteen years old children, admitted because of congenital heart disease

Group 7 Adults, nursing staff or family members of diagnosed or suspected cases of CMV infection

Virological techniques used in the diagnostic tests for CMV were those previously described (2) CMV was looked for in urine and/or throat specimens and serological diagnosis was made by complement fixation tests (CFT) with paired acute and convalescent sera or in newborn infants with repeated sera collected at two-month intervals Some patients were studied by both virus isolation and serological methods sometimes only one of the diagnostic procedures was applied

Isolation and identification of CMV Throat swabs collected in 1 5 ml of virus transport medium (gelatine lactalbumin hydrolysate yeast extract medium with penicillin streptomycin and nystatin) and equal parts of urine and transport medium respectively were inoculated within a couple of hours after collection into diploid human embryonic fibroblasts Each tissue culture tube was inoculated with 0 5 ml and

7 McKusick V A *Heritable disorders of connective tissue* C V Mosby Co Saint Louis 1966 3rd edition
8 Manley G & Hawksworth J Diagnosis of Hurler s syndrome in the hospital laboratory and the determination of its genetic type *Arch Dis Child* *41* 91 1966
9 Öckerman P A Glycogen storage disease in Sweden *Acta Paediat Scand* Suppl *160* 1965
10 — A technique for the enzymatic diagnosis of glycogen storage disease on very small tissue specimens *Acta Paediat Scand* *57* 105 1968
11 — Lysosomal acid hydrolases in the liver in gargoylism Deficiency of 4 methylumbelliferyl β galactosidase *Scand J Clin Lab Invest* *22* 142 1968
12 Soderstrom N *Fine needle aspiration biopsy* Grune & Stratton New York 1966
13 Teller W M Burke E C Rosevear J W & McKenzie B F Urinary excretion of acid mucopolysaccharides in normal children and patients with gargoylism *J Lab Clin Med* *59* 95 1962

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Case 1



Case 2

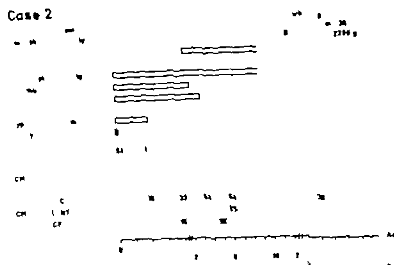


Chart 1. Relevant data in two children with congenital CMV infection

performed as proper serum samples were not obtained. In two of the seronegative cases the CMV suspected agent was found to be herpes simplex. On four occasions adenovirus was isolated, this virus however causing a different cytopathogenic pattern in the fibroblasts.

Distribution of CMV antibodies

Complement fixing titres of single sera from patients of all ages were used to study the distribution of CMV antibodies in different groups of patients. Previously published data on the antibody pattern of 242 patients of different ages selected at random in Karolinska Sjukhuset (1) were included in this material. The results of the in all 811 human sera studied with CFT are recorded in Fig. 1.

The intention was to compare the CMV antibody pattern in children with congenital heart diseases and central nervous system diseases with that of the rest of the patients. Children with heart diseases were found to have an equivalent pattern to that of the main group and were therefore included, while a higher percentage of seropositive individuals was found at all ages among patients with neurological diseases (Fig. 1). This difference however was not significant and from this material no conclusions can be drawn as to the role of CMV in the aetiology of neurological diseases.

Table 1 *Incidence of CMV infection in different groups of patients*

Group	Number of patients tested					Total patients tested	CMV positive patients
	By virus isolation from			By serology			
	Throat	Urine	Throat + urine	CFT + isol	CFT only		
1 (newborn infants jaundiced)		105	13	13[a]	10	128	0
2 (0-6 mo infections suspected)		29	19	21	14	62	2
3 (7 mo-15 yr acute infections)		24	60	18	12	96	2
4 (7 mo-5 yr pseudocroup)	194					194	0
5 (7 mo-15 yr cerebral damage)	4		30			34	0
6 (7 mo-15 yr cong heart disease)	11	2	44			57	0
7 (well adults contacts)	10	12	14	6	6	42	0
Total						613	4

[a] For obvious reasons the figures in this column are not included in the totals

the maintenance medium was changed at intervals of one week Inoculated cultures were examined once a week for two months and positive cultures were passaged by transference of infected cells to new tissue cultures After four passages CMV could usually be transferred by the fluid medium Isolated strains which caused CMV typical cytopathogenic effects in fibroblasts were tried in HeLa and GMK cells and when negative were checked by neutralization tests (NT) against paired acute and convalescent sera from patients with previously diagnosed CMV infection

Serological tests In CFT and NT the Ad 169 strain of CMV was used Paired acute and convalescent sera were tested simultaneously as were sera from a mother and her newborn infant as well as repeated sera from the infants CFT was used for primary screening A fourfold rise in titre was taken as a significant change All sera were tested at two fold dilution steps 1/2 being the lowest dilution When CMV infection was diagnosed by CFT NT was done

RESULTS

Prevalence of CMV infection

Virological investigation for CMV infection was performed in 613 hospital admissions four positive cases being found Two of these were diagnosed by virus isolation and serology and two by serology only The incidence of CMV infection in the different groups of patients is given in Table 1

Two of the positive cases were infants representing congenital CMV infection and two belonged to Group 3 i e older children with symptoms of acute infection

Congenital infection As previous reports on congenital CMV infections are now numerous relevant data on our two cases are summarized in Chart 1

Acquired infection Two children 4 and 9 years of age had CMV infection running the course of an acute acquired infectious disease These patients also had severe chronic diseases and were both on treatment with cortisone Relevant data on these cases are summarized in Chart 2 A detailed case description will be published elsewhere

Virological diagnosis of CMV infection

In virus isolation trials cytopathogenic effects with focal lesions suggestive of CMV were found in 14 patients Three of them (and one with negative isolation trial) were seropositive for CMV (rise in titre from acute to convalescent phase) and in two of the three cases CMV could be subcultured In 12 patients subpassage of CMV suspected agent could not be accomplished Serological investigations for CMV were positive in two and negative in 8 of these 12 cases In two cases serology was not

Table 2 *CMV antibodies in mothers and their infants*
Non CMV infected subjects

	Mothers at delivery	Infants At birth	After 1–3 months	After 4–6 months
Number of seropositive subjects (CFT 4-64)	23	19/19	4/14	0/6
Number of seropositive subjects (CFT < 2)	11	11/11	5/5	—

Denominator indicates total number tested in each group

Maternal CMV antibody titre

Results of studies on transplacental passage of CMV antibodies and the persistence of maternal titres are given in Table 2

In many cases transmitted antibodies disappeared within a couple of months and were not found to persist after 4 months

DISCUSSION

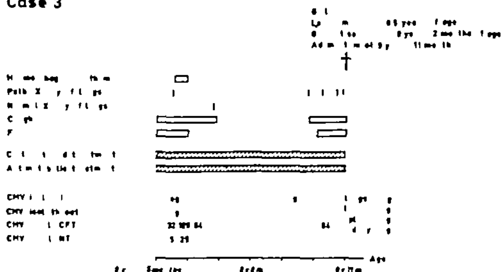
The epidemiology of CMV infection has been studied from the point of view of virus excretors in many differently selected hospital materials Row *et al* (15) found healthy newborns and adults to be negative but demonstrated relatively high CMV isolation rates (28%) among children 8 months–4 years of age institutionalized for socio-economic reasons These results were in principle confirmed by Hanshaw *et al* (7) who furthermore demonstrated a high incidence of excretors among children who were contacts to patients excreting CMV Adult contacts were negative Stern (18) in a study of unselected hospital admissions in London found that 10% of children between 2 months and 5 years old were excreting CMV Only one virus excretor aged 6 years was found among 575 older children and adults

Many of the virus excreting children reported by other authors were not overtly ill but on retrospective examination showed different symptoms particularly liver dysfunction respiratory diseases and minor illnesses The majority recovered completely and Stern stresses that, contrary to currently held views this goes also for infections in the early neonatal period On the other hand Stern reports a patient who developed a severe brain injury after an "inapparent congenital CMV infection

In the present study CMV infection was demonstrated in four out of 613 patients and the positive cases were all overtly ill Two were infants and had all the classical symptoms commonly associated with neonatal cytomegalic inclusion disease The two older children had acute pneumonia and suffered from chronic diseases The four patients were all treated with cortisone No subclinical cases were found The incidence of CMV infection in this material is in agreement with the findings of Hanshaw *et al* (7) in healthy and hospitalized children (1 per cent isolation rate) but is low compared to findings in chronically institutionalized patients (7 15) and also compared to the figures of Stern (18) for hospitalized children between two months and five years of age

The laboratory technique for virus isolation is of interest in this connection CMV is an unstable agent Some virologists have tried to increase the sensitivity of the virus isolation technique by using ultracentrifuged urine specimens This was not done here as it would cause a delay of the inoculation procedure and increase the risk of virus inactivation A laboratory routine requiring accomplished serial subcultivations of isolated CMV strains may lead to the loss of labile low titre virus in some positive cases However the sole criterion of typical cytopathogenic effect in isolation trials was not considered quite safe and specific for the diagnosis of CMV nor was positive cytology alone As a rule typical cytopathogenic agents could be subcultured but uneventful subpassages of CMV were found to occur Occasionally CMV suspected agents were identified as herpes simplex The two patients with

Case 3



Case 4

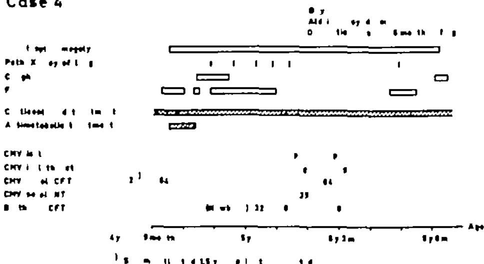


Chart 2 Relevant data in two children with acquired CMV infection

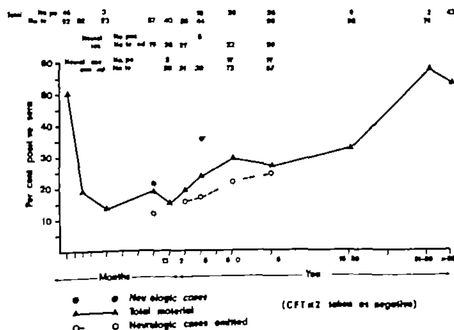


Fig 1 Distribution of CMV antibodies in different age groups of 811 patients

single sera be considered to be a significant diagnostic criterion

ACKNOWLEDGEMENT

We wish to express our appreciation to Mrs Gunilla Hedstrom and Mrs Eva Olsson for competent technical assistance

REFERENCES

1 Andersson J P & Stern H Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis *Brit Med J I* 672 1966

Carlstrom G Virologic studies on cytomegalic inclusion disease *Acta Paediat Scand 54* 17 1965

3 Carlstrom G Alden J Belfrage S Hedenstrom G Holmberg L Nordbring F & Sterner G Acquired cytomegalovirus infection *Brit Med J II* 5 1 1968

4 Carlstrom G Belfrage S Ohlsson N M & Swedberg J Cytomegalovirus infection complicating open heart surgery *Scand J Thor Cardiovasc Surg 2* 57 1968

5 Hanshaw J B Congenital and acquired cytomegalovirus infection *Pediat Clin N Amer 13* 279 1966

6 — Cytomegalovirus complement fixing antibody in microcephaly *New Eng J Med* 275 476 1966

7 Hanshaw J B Betts R F Simon G & Boynton R C Acquired cytomegalovirus infection *New Eng J Med* 272 602 1965

8 Jordan A G & Tobin J O H Cytomegalovirus infection in the adult *Lancet II* 615 1967

9 Irvin E & Kenny G E Cytomegalic inclusion disease of infancy *Pediatrics 38* 957 1966

10 Klemola E & Kaariainen L Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis *Brit Med J II* 1099 1965

11 Klemola E Kaariainen L von Essen R Haltia K Koivuniemi A & von Bonsdorff C H Further studies on cytomegalovirus mononucleosis in previously healthy individuals *Acta Med Scand 182* 311 1967

12 Krech U Jung M Jung F & Sonnabend Ch Virologische und klinische Untersuchungen bei konnatalen und postnatalen Cytomegalien *Schweiz Med Wschr* 98 1459 1968

13 Lamb S G & Stern, H Cytomegalovirus mononucleosis with jaundice as presenting sign *Lancet II* 1003 1966

14 Medearis, D N Jr Observations concerning human cytomegalovirus infection and disease *Bull Hopkins Hosp 114* 181 1964

15 Rowe W P, Hartley J W Cramblett, H G & Mastrota F M Detection of human salivary gland virus in the mouth and urine of children *Amer J Hyg 67* 57 1958

16 Rowe W P Hartley J W Waterman, S Turner H C & Huebner R J Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids *Proc Soc Exp Biol 92* 418 1956

17 Rasmussen V & Sarkis P Cytomegalovirus hepatitis *Lancet II* 772 1967

18 Stern, H Isolation of cytomegalovirus and clinical manifestations of infection at different ages *Brit Med J I* 665 1968

19 Stern, H & Elek S D The incidence of infection with cytomegalovirus in a normal population *J Hyg 63* 79 1965

20 Toghill P J Bailey M E Williams R, Zeegen R & Brown R Cytomegalovirus hepatitis in the adult *Lancet I* 1351 1967

21 Weller T H & Hanshaw J B Virologic and clinical observations on cytomegalic inclusion disease *New Eng J Med* 266 1233 1962

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positive CMV isolations were both on treatment with cortisone a procedure which may improve the conditions for positive isolations In the present cases it seems possible that cortisone has been life saving in the acute CMV incidences whereas it may have provoked clinical CMV infection in the chronically treated patients

From the present study previously published experiences (3 4) and unpublished data —in all comprising 26 positive cases—it may be concluded that in our hands complement fixation tests of paired acute and convalescent sera seem to be the routine diagnostic method of choice for CMV infection It is preferable also to virus isolation because of the instability of the virus the particular relations between CMV and its host cell and the chronicity of the virus infection Combined virus isolation and serology tests are of course preferable

Serological diagnosis alone may be accomplished also in newborn babies as maternal antibodies are eliminated rather rapidly from the infant s blood As shown in Table II most such antibodies were eliminated in 1–3 months and after 4–6 months no persisting titres were found Therefore a neonatal CMV titre persisting for more than 4 months is to be considered important and should lead to an attempt to isolate virus Infants as well as older patients have responded to CMV infection with a significant rise in antibody titre and in all diagnosed cases there has been a long lasting persistence of the complement fixing CMV antibodies Not even in the patient followed for five years was a decrease in titre recorded This trend has been demonstrated also in our previous publications (4) and differs from the pattern of immune reaction in many other infectious diseases particularly regarding CF antibodies The chronicity of CMV infection may have a bearing upon this fact

One of our intentions stimulated by previous reports (6) was to investigate whether the demonstration of CMV antibodies in single sera could be used as a significant diagnostic criterion in patients less than five years of age This was not found to be the case since rather many of the children of this age were found to be seropositive The present material was selected among hospitalized and not chronically institutionalized children The pattern of their immune response (Fig 1) indicates a tendency to an early appearance of CMV antibodies 12 5 and 23 per cent of children at months and five years of age respectively were seropositive Corresponding figures reported by Hanshaw (6) were 2 6 and 63 per cent and by Stern & Elek (19) 4 per cent To investigate more thoroughly whether this reflects a real epidemiological difference in Sweden or whether the present clinical material is selected virus isolation and serological studies for CMV are in progress on children of different ages attending a well baby clinic One may speculate whether the rather high rate of seropositive children in this study may have a bearing upon the comparatively low rate of virus isolation

SUMMARY

The incidence of CMV infection was studied in 613 patients of various ages in Stockholm Four positive cases were found two infants with congenital infection and two older children who also suffered from other chronic diseases and were treated with cortisone

Complement fixation tests of paired acute and convalescent sera have in our hands been the routine diagnostic method of choice for CMV infection Serological diagnosis alone may be accomplished also in newborn babies as the maternal antibodies were found to be eliminated rather soon from the infants blood

The distribution of CMV antibodies at different ages and in different clinical groups of patients was studied by CFT in 811 human sera There was no significant difference between the various clinical groups A relatively high percentage of children less than five years of age were found to be seropositive (12 5–23 %) Therefore not even in small children can the demonstration of CMV antibodies in

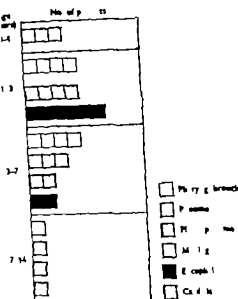


Fig. 1. Clinical diagnosis of adenovirus infections in relation to age.

of complement were employed in the test. Serum specimens were also tested in many cases with antigens of influenza A 2 and B, strains parainfluenza 1, 2 and 3, respiratory syncytial, herpes simplex, cytomegalo, reo, ornithosis, polio 1, 2 and 3, Coxsackie A 7, Coxsackie B 1-5, and mycoplasma pneumoniae.

RESULTS

Clinical picture

From January to July 1967, 32 children with an infection produced by adenovirus type 7 were treated in the Department of Paediatrics, University of Oulu. Adenovirus type 7 was isolated from these patients and/or they had a significant (4 fold or higher) rise in titres.

The principal clinical diagnoses were

pharyngobronchitis	10
pneumonia	11
pleuropneumonia	2
meningitis	1
encephalitis	7
carditis	1

Fig. 1 shows the principal clinical diagnosis of these patients in relation to age. Fig. 2 presents the monthly incidence of the adenovirus infections and of encephalomeningitis.





Fig. 2. Monthly incidence of patients with (*a*) adenovirus infection and (*b*) adenovirus encephalomeningitis treated in the hospital.

Fig. 3 illustrates the typical symptoms and findings in the patients with adenovirus encephalomeningitis. As can be seen, the prevalent symptom was fever.

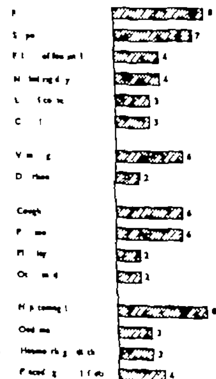


Fig. 3. Clinical picture of adenovirus encephalomeningitis.

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ENCEPHALOMENINGITIS IN CHILDREN ASSOCIATED WITH AN ADENOVIRUS TYPE 7 EPIDEMIC

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Adenoviruses usually cause widespread epidemics of acute respiratory disease in military recruits (16) and limited outbreaks among children Other adenoviral diseases are ceratoconjunctivitis and pneumonia The latter is a serious and sometimes fatal adenovirus disease in children (18 23) More unusual adenovirus disease are myocarditis nephritis and meningoencephalitis (2 3 4 15 25)

Encephalitis associated with adenovirus infection was first described by Lelong *et al* (15) who isolated adenovirus type 7 from the cerebrospinal fluid and brain tissue of fatal encephalitis in a child Association of adenovirus type 7 with encephalitis has also been reported by Gabrielson *et al* (8) and Jansson *et al* (9) Usually the encephalitis is only one of the many symptoms caused by severe generalized adenovirus infections in children and encephalitis occurs more frequently than meningitis In addition to type 7 adenovirus types 1 2 3 6 and 12 have been isolated from patients with encephalitis or meningitis (5 13 14 20 22) Stenger *et al* (24) reported an epidemic caused by an unidentified adenovirus type in a children's home where 15 of the 60 children infected developed encephalomeningitic symptoms

The present report describes 8 cases of encephalomeningitis or meningitis in infants and children associated with an adenovirus type 7 epidemic in Northern Finland, Pohjanmaa

MATERIALS AND METHODS

Study population

The clinical material consisted of all children ([illegible] cases) with an adenovirus type 7 isolation and/or increase in adenovirus CF antibody titre admitted to the Department of Paediatrics University of Oulu, between January and July 1967 The children came from different families living in the city Oulu and ten surrounding municipalities

Virus isolation

Throat and anal swab specimens were inoculated into duplicate cultures of HeLa U (continuous line of human amnion cells) and primary monkey kidney cells These cell cultures were maintained in Eagle's MEM with 5 inactivated calf serum Eagle's MEM with 2 5 chicken serum and medium no 199 with 0 5% bovine plasma albumin respectively Cell cultures inoculated with anal specimens were incubated at 37 C and with throat specimens at 34 C for 14 days The inoculated cultures were examined 3 times a week and the media were changed every 4 to 5 days Subcultures were made if viral degeneration of the cell was suspected Hemadsorption tests with human type 0 erythrocytes were done in monkey kidney cultures For the identification of virus isolates cultures with completely degenerated cells were frozen and thawed 5 times and the supernatant was used in the identification tests Adenovirus isolates were first identified by CF technique using paired human sera with a known rise in CF antibody titre Further typing of adenovirus isolates was done by a hemagglutination inhibition test using type specific rabbit antisera against prototype strains of adenoviruses

Serological tests

Antibody determinations of paired serum specimens were carried out with complement fixation test according to a slightly modified micromethod of Sever (21) Adenovirus antigen was prepared in HeLa cells with type 5 virus Four antigen units and 2 full units

Table 2 *Cerebrospinal fluid and EEG in patients with encephalomeningitis*

Case no	Age	Sex	Clinical symptoms	Cerebrospinal fluid: Specimen taken days after admission to hospital	Cells in mm³	Sugar (mg/100 ml)	Prot (mg/100 ml)	Electroencephalography: Taken days after admission to hospital	Findings
1	2 y	M	Fever pneumonia stupor nuchal rigidity	9 0	1 0	86 48	18 12	2 11	Pathological Normal
2	1 y 4 m	M	Fever pleuropneumonia oedema hepatomegalia haemorrhagic diathesis loss of consciousness convulsions death	0 10	0 0	56 52	18 118		
3	8 y	M	Fever pharyngobronchitis nuchal rigidity	0 6	560 340	61 53	93 220		
4*	1 y 3 m	F	Fever pleuropneumonia oedema hepatomegalia haemorrhagic diathesis loss of consciousness convulsions death						
5	1 y 1 m	M	Fever pneumonia oedema hepatomegalia confusion irritability	0	0	63	26	2 22	Pathological Pathological
6	1 y 5 m	F	Fever oedema hepatomegalia stupor fulness of fontanel nuchal rigidity irritability	0	3	56	16	1	Pathological
7	3 y 11 m	F	Fever pleuropneumonia oedema hepatomegalia loss of consciousness convulsions death	1	15	41	16		
8	2 y	M	Fever pneumonia oedema hepatomegalia confusion nuchal rigidity	1	2	59	—		Pathological

Table 1 *Virus isolations and antibody studies in patients with encephalomeningitis*

Case no	Type of specimen	Specimen taken: Days after onset of illness	Days after admission to hospital	Adenovirus isolated	Adenovirus CF antibody titre
1	Throat swab	16	7	+	
	Serum	12	3		1/16
	Serum	26	17		1/64
2*	Anal swab	17	3	+	
3	Throat swab	16	9	+	
	Serum	7	0		1/4
	Serum	21	14		1/16
4	Serum	9	1		1/8
	Lung (in the autopsy)	9	1	+	
5	Throat swab	6	1	+	
	Anal swab	6	1	+	
	Serum	6	1		1/16
	Serum	19	14		1/256
	Serum	27	22		1/256
6	Throat swab	7	1	+	
	Anal swab	7	1	+	
	Serum	7	1		1/64
	Serum	16	10		1/256
7*	Anal swab	3	1	+	
	Lung (in the autopsy)	9	7	+	
	Serum	3	1		1/4
8	Throat swab	9	2	+	
	Anal swab	9	2	+	
	Serum	9	2		1/64
	Serum	17	10		1/128

Fatal disease

Convulsions occurred in three patients stupor in seven loss of consciousness in no less than three All patients had respiratory symptoms Six had roentgenologically verified pneumonia, and three additional pleurisy Hepatomegalia and oedema were very often part of the clinical picture Haemorrhagic diathesis appeared in the most severe cases

Virological findings

Adenovirus type 7 were isolated in all cases (Table 1) in two (cases 1 3) from the nasopharynx only in two (cases 2 7) from the faeces and in three (cases 5 6 8) from both specimens In one patient it was isolated only from lung tissue taken on autopsy (case 4) No other viruses were isolated from these specimens A fourfold of greater rise in adenovirus CF antibody titres was seen in six patients In the fatal case (case 2) no specimens for adenovirus antibodies were taken but the finding on the autopsy was similar to that of the fatal infections associated with adenovirus reported in literature (3 15 17 20) In one fatal case (case 4) there was time for only one specimen to be taken

No rise in CF antibody titres of influenza A 2 and B mumps parainfluenza 1 2 and 3 respiratory syncytial herpes simplex cytomegalo reo ornithosis polio 1 2 and 3 Coxsackie A 7 Coxsackie B 1–5 and mycoplasma pneumoniae was found in the seven patients

Clinical laboratory findings

Cerebrospinal fluid findings are presented in Table 2 Pleocytosis was noted in two patients and protein increased in two ones The ESR exceeded 20 mm/h in five patients The

ity cannot be judged Since however no less than one *fourth* of the cases requiring admission to hospital had symptoms of the central nervous system it would seem that the rate of meningoencephalitis and meningitis associated with this epidemic was remarkably high

In the present cases adenovirus type 7 was isolated from the nasopharynx or rectal mucosa and in two also from lung tissue No virus isolations from the cerebrospinal fluid were carried out owing to the distant location of the virus laboratory Adenovirus type 7 has only once been isolated from cerebrospinal fluid and brain tissue (15)

Virus encephalitis may either be directly caused by the virus or follow after infection (12) Two of the present patients had a recent history of measles or chickenpox In these cases the encephalitis may have been post infectious and children may have had the respiratory disease caused by the adenovirus Furthermore epidemic adenovirus type 7 often occurs in closed communities (16) It is therefore theoretically possible that the present patients contracted their adenovirus infection in the hospital while some other agents had caused the initial disease This assumption is contradicted in many cases by the fact that the adenovirus could be isolated both from the nasopharynx and the rectal mucosa as early as 1–2 days after admission The adenovirus CF antibody titre which on admission were distinctly above the average level also suggest an infection already contracted at home especially since antibodies to other tested viruses had not increased It seems therefore most probable that the meningoencephalitis at least in the majority of cases was caused by adenovirus type 7

The prognosis of adenovirus encephalomeningitis is not very well known The mortality rate is very high in the present series 38 per cent and in Stenger's series 26 per cent (24) Although the clinical picture is severe the patients who survive the acute phase of the disease contract no irreversible lesions (1 9 14 24)

SUMMARY

Of the 32 children with an adenovirus type 7 isolation and/or a significant rise in adenovirus CF antibody titre treated in the hospital seven had meningoencephalitis and one meningitis The encephalomeningitis was associated in each case with respiratory illness six had pneumonia and three additional pleurisy The patients with encephalomeningitis were seriously ill three were unconscious three cases were fatal The prognoses of those surviving was apparently good according to a short observation period

REFERENCES

1 Bebe M Chany C Habib E C, Molkhou P Robinson J Soulas P & Lelong M Epidemie familiale de maladie à adenovirus type 7 avec manifestations encéphalitique et sanguines *Arch Franc Pediat* 21 87 1964

2 Cabanel G Voog R & Phélip X Pericardite aique avec reaction de deviation du complément positive aux adeno-virus *J Med Lyon* 47 83 1966

3 *Chany C Lepine P Lelong M* Le Tan Vinh Satge P & Virat J Severe and fatal pneumonia in infants and young children associated adenovirus infections *Amer J Hyg* 67 367 1958

4 Dahl M Sumila S & Salmi A Myocarditis associated with adenovirus type 7 infection To be published

5 Ehrlieb M & *Fodor* Rankle *G* Adenovirus infection (type 12) as a cause of meningoencephalitis *Z Kinderheilk* 87 275 1963

6 *Forssell P Halonen H* Stenstrom *R* Jansson F & Wager O An adenovirus *epidemic* due to types 1 and 2 *Ann Paediat Fenn* 8 35 1962

7 Gabrielson M O Joseph C & *Huang* G D Encephalitis associated with adenovirus type 7 *occurring in a family outbreak J Pediat* 68 142 1966

8 Gibbs F A & Gibbs E L *Atlas of electroencephalography* vol 3 *Neurological and psychiatric disorders* Addison Wesley Publ Comp London 1964

9 Jansson E Wager O Forssell P & Stenstrom R Epidemic occurrence of adenovirus type 7 infection in Helsinki *Ann Paediat Fenn* 8 24 1962

10 Jen K P *Tao Y Lin Y C* & Wang H Y The role of adenovirus in the etiology of infantile pneumonia and pneumonia complicating measles *Chin Med J* (Peking) 81 141 1962

11 Kaski U & Jouppila, R., Acute cerebellar ataxia associated with adenovirus type 7 infection To be published

haemoglobin was less than 10 g/100 ml also in two patients The blood leucocytes were less than 5000 per mm³ blood in the acute phase of the disease in three patients while in others the leucocyte counts were normal or slightly increased Three patients showed neutrophilia No monocytosis was noted The blood thrombocytes were less than 100 000 in eight patients, but less than 50 000 in only one patient

EEG findings

In four patients in the acute phase of the disease examined with electroencephalography pathological findings compatible with encephalitis (8) were recorded (Table 2) A follow up EEG was carried out on two patients in the convalescent phase in one the finding was normal while in the other the changes had diminished compared with the first examination

Prognosis

In three cases the disease was fatal In one case the encephalitis was the direct cause of death while in two the disease was complicated by severe thrombocytopenic haemorrhagic diathesis Treatment with prednisolone was attempted but with no observable effect on thrombocytopenic or haemorrhagic diathesis Autopsy was carried out on all three and the findings agreed with an adenovirus infection The histological examination of the brain revealed lymphocyte infiltration in one patient and only oedema and diffuse cellular changes in two The most striking findings were in the lungs which were heavy solid in consistency and dark red in colour Microscopically the predominant feature was a diffuse interstitial pneumonitis, characterized by a moderate mononuclear cell infiltrate and hyperplasia of the alveolar lining cells Many of these alveolar cells were greatly enlarged with large nuclei containing one or multiple eosinophilic inclusions This picture is similar to that of fatal infections associated with adenovirus type 7 reported in literature (3 15 17 20)

All patients were treated with antibiotics They were hospitalized for 3–48 days five patients for 3–14 days two for 14–21 days one more than 21 days

DISCUSSION

Pharyngoconjunctival fever in children and a mild infection of the upper respiratory tract in adults are usually considered the most typical clinical manifestations of an adenovirus infection According to the present observations it would seem however that adenovirus type 7 may in early infancy produce a very severe clinical picture Encephalomeningitis was unexpectedly frequent in this age group Of the 32 children with adenovirus infection treated in our hospital, no less than seven showed the clinical picture of encephalomeningitis and one that of meningitis It seems that encephalomeningitis is often associated with the adenovirus type 7 infection particularly in the second and third year of age School age children have neurologic symptoms distinctly less often in connection with this virus infection (9) In these cases the clinical picture seems to be one of the meningitis (9) In older children adenovirus type 7 may cause isolated cerebellar ataxia (11) myo- or pericarditis (2 4 25)

The special clinical symptoms which may occur in infection caused by adenovirus type 7 are considerable oedema hepatomegalia and in the most severe cases thrombocytopenic haemorrhagic diathesis These symptoms if present suggest a very severe infection (1) Leucocytosis and neutrophilia may belong to the clinical picture (9 23) as may monocytosis (6 9) Adenovirus infection has often been found to follow a bacterial or viral infection (10 17 19) especially measles In two of the present patients with encephalomeningitis some other virus infection (measles and chickenpox) preceded the adenovirus infection None of the present patients however revealed any immunologic defect The serum electrophoreses and immunoglobulins were normal in all cases

The extent of the epidemic in the province was not known and therefore the rate of encephalomeningitis calculated from total morbid

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JUVENILE HYPOTHYROIDISM WITH TESTICULAR ENLARGEMENT

Z LARON M KARP and L DOLBERG

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Though hypothyroidism is one of the more frequently encountered endocrine disorders in the paediatric age group (14) little documentation has been made of the development of the gonads when thyroxine secretion is made quate Longstanding prepubertal hypothyroidism has been described as resulting in delayed maturation of the testicles in boys (1 8) and delayed puberty in girls (7 12) According to these investigators this condition which was accompanied by decreased excretion of urinary gonadotrophins and sex hormones was corrected by treatment with thyroid preparations

In contrast to the latter clinical picture hypothyroidism has also been known to be associated with precocious sexual development This condition was first reported by Kendle (6) in 1905 but it was only in 1955 that a report by Bergstrand (2) drew renewed attention to it It is noteworthy that in a total of 17 cases reported since then from various countries (4 5 9 10 13) only 3 have been males (in one of these the hypothyroidism was associated with Down s syndrome (9))

In the present paper we report on four more male patients with hypothyroidism and testicular enlargement

CASE REPORTS

Patient 1 M Y was referred at the age of 6 / years because of growth and mental retardation Both parents and three other children were healthy and there was no family history of thyroid disease Pregnancy and delivery were normal From the age of 3 years the parents had noticed obesity sluggishness and a decreased growth rate but he did not receive any treatment



Fig 1 Patient M Y at the age of 6 /₁₂ years Note short stature for age plumpness and large testicles without pubic hair

12 Kennedy C & Wanglee P Encephalitis A variable syndrome in response to viral infection *Pediat Clin N Amer 14* 809 1967
13 Kibrick S Meléndez L & Enders F J Clinical associations of enteric viruses with particular reference to agents exhibiting properties of the ECHO group *Ann NY Acad Sci 67* 311 1957
14 Kackell M Y Encephalomyelitis infolge Adenovirus Typ-3 Infektion *Mschr Kinderheilk 112* 99 1964
15 Lelong M Lépine P Alison F Le Tan Vinh Satgé P & Chany C La pneumonie a virus du groupe A P C chez le nourrisson isolement du virus Lesl lésions anatomohistologiques *Arch Franc Pediat 13* 1092 1956
16 Mantyjarvi R Adenovirus infections in servicemen in Finland *Ann Med Exp Biol Fenn 44* Suppl *4* 1966
17 Nahmias A J Griffith D & Snitzer J Fatal pneumonia associated with adenovirus type 7 *Amer J Dis Child 114* 36 1967
18 Osvath P & Toth M Eine durch Adenovirus 7 verursachte Kindergarten Epidemie *Arch Kinderheilk 172* 286 1965
19 Patzer von H & Braun W Die Adenovirus pneumonie Symptomatologie und Klinik *Munchen Med Wschr 107* 1985 1965
20 Sattelkau G Der Nachweis einer Adenovirusinfektion (Typ 3) bei schwer bzw todlich verlaufener Meningo Enzephalitis *Arch Kinderheilk 170* 174 1965
21 Sever J L Application of a microtechnique to viral serological investigations *J Immun 88* 3?? 1962
22 Sohier R Chardonnet Y & Prunieras M Adenoviruses Status of current knowledge *Prog Med Virol 7* 253 1965
23 Spreer F Tauchnitz Ch Schmidt L Schmidt J Keil R & Cotteschling C Todliche Adenoviruspneumonie im Kleinkindalter Bericht über zwei eigene Beobachtungen *Arch Kinderheilk 172* 284 1965
24 Stenger K Kaffanke V & Schauerhammer O Über eine Adenovirus Epidemie mit schwerer Encephalopathie *Mschr Kinderheilk 113* 4?? 1965
25 Van Zaane D J & Van der Veer J Some peculiar clinical symptoms in children with an adenovirus infection *Presse Med 70* 10?1 196?

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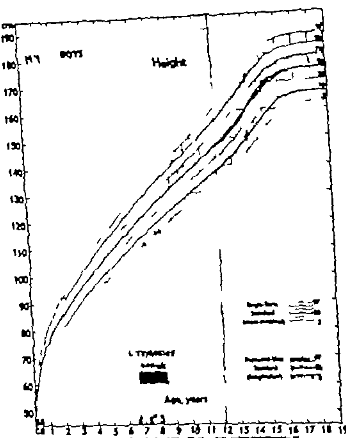


Fig. 4. Patient M. Y. Growth chart denoting good response of linear height to thyroid therapy.

one year of treatment there was no change in the testicular volume. The child is mentally retarded and attends a special school.

Patient 2. J. S. was referred to us at the age of 14 years because of mental retardation. There was no family history of thyroid disease. His motor development was slow and at the age of 3 years he had been diagnosed as suffering from hypothyroidism. H. was subsequently treated irregularly with thyroid preparations until 6 months before he came to us. During these 6 months he had become obese, sensitive to cold and constipated. Physical examination revealed a mentally retarded boy. The skin was pale and cold. There was bilateral pretibial myxedema. The thyroid gland was not palpable. His height 144 cm, corresponding to a height age of 11 years. The skeletal age was 12 years. Axillary hair was absent but pubic hair was normal for his age. The penis was of normal size but the testes were moderately enlarged with a volume of 15 ml each. Laboratory data revealed: Hemoglobin g/100 ml, erythrocytes 3.2 10^6/mm, cholesterol 4 mg/100 ml, PBI 2.1 µg/100 ml. Thyroid radioiodine uptake was 4.5 after 4 hours and did not change after TSH injection (700 I.U.). Urinary 17-ketosteroids were 1.5 mg/24 hours and the 17-hydroxycorticosteroids 4.4 mg/4 hours. The metopyrone and ACTH tests were normal. Urinary gonadotrophins were 3 M.U.U./24 hours. X-ray of the skull showed an enlarged sella.

During 5 years of treatment with thyroid extract or l-thyroxine the boy achieved normal height (168 cm at the age of 20 years) and a normal sexual development. There was a reduction in the size of the testes and at the age of 20 years their volume was 20 ml which is within the normal range for this age. Testicular biopsy was not performed. The young man is mentally retarded and institutionalized.

Patient 3. A. W. was referred to us at the age of 17 years because of mental retardation. Both parents and two brothers aged 18 and 22 years are healthy. Another younger brother is described as case 4. The parents claim that the boy had already shown signs of motor and mental retardation in early infancy.

Physical examination revealed a boy with a proportional body build. Body height was 160 cm corresponding to a height age of 14 years. His epiphyses were closed. He had no goiter.

Pubic and axillary hair were of normal appearance. The penis length was 9.5 cm but the testes were abnormally large with a volume of over 50 ml each. X-ray of the skull was normal. Laboratory findings were: PBI 3.8 µg/100 ml, BEI 2.5 µg/



Fig 2 Patient M Y Roentgenogram of the skull showing an enlarged sella turcica

Physical examination revealed a pale moderately obese child (Fig 1) The skin was dry and dental development was retarded The thyroid gland was not palpable Linear height was 103 5 cm corresponding to a height age of 4 /₁₂ years The skeletal age was 1 year Pubic and axillary hair were absent Penile length was 3 3 cm Both testes were firm and enlarged for his age (15) measuring 7 8 ml in volume (Fig 1) Laboratory data revealed Hemoglobin 10 3 g/100 ml erythrocytes 3×10^6/mm cholesterol 280 mg/100 ml PBI 1 8 μg/100 ml Thyroid radioactive ^{131}I uptake was 10 5 after 2 hours without a further rise after 24 hours nor after TSH administration 200 I U in 2 injections Urinary 17 ketosteroids were 0 4 mg/24 hours and 17 hydroxycorticosteroids 2 3 mg/24 hours No gonadotropins were detected by bioassay in several 48 hour urine collections Plasma gonadotropins were as follows FSH—24 2 ng/ml (normal adult value 17 4 ± 3 8 ng/ml) LH—1 5 ng/ml (normal adult value > 1 68 ng/ml) (3) X ray of the skull showed an enlarged sella (Fig 2) Biopsy of the testes was performed under general anaesthesia histological examination revealed beginning spermatogenesis with a few spermatocytes and spermatids and a few Leydig cells (Fig 3) a picture consistent with that of beginning puberty

Following therapy with *l* thyroxine in a dose of 0 05 mg/day there was a rapid advancement in growth velocity (Fig 4) and in skeletal age The child became more active and lost weight During

Plasma gonadotropins were kindly measured for us by Dr P Franchimont Department of Clinical Medicine and Medical Pathology University of Liege Belgium by a radioimmunoassay method



Fig 3 Patient M Y Histological appearance of testicular biopsy at age 6 / The early spermatogenesis and Leydig cells are consistent with early puberty HE × 40

not certain that he took his medication. His height and secondary sexual development progressed normally. Testicular biopsy performed at the age of 15 1/12 years showed a normal histological picture for this age.

A summary of the pertinent clinical data of the 4 patients and the other 3 male patients reported in the literature are presented in Table 1.

DISCUSSION

It has yet to be clarified why primary hypothyroidism which remains untreated for long periods of time should affect sexual maturation in opposite ways, i.e. it may result in a lack of sexual maturation and a failure of sexual organs to develop, or in precocious puberty and an abnormal increase in the size of the gonads. The latter phenomenon is more frequently encountered in girls.

Van Wyk & Grumbach (13) suggested that the precocious puberty might have resulted from an overlap mechanism of the hormones TSH and the gonadotrophins (or their releasing factors) at the hypothalamic or pituitary level. Among our 4 patients, only in patient 1 were we able to prove the existence of elevated plasma FSH and LH levels (measured by radioimmunoassay) in the absence of urinary gonadotrophins (measured by bioassay). The other patients were too old to draw any conclusions from the moderately increased gonadotrophin output found in the urinary assay. Even so, the finding of increased plasma FSH and LH in the one patient tested fails to indicate the exact mechanism involved. The enlarged sella turcica found in several of the patients could have resulted from hypertrophy of the thyreotropin cells alone.

It is noteworthy that the most common manifestation of precocious sexual development in boys with hypothyroidism is an enlargement of the testicles. This is not particularly surprising since enlargement of the testes is the first sign of puberty in boys (11). Considering the fact that in the older patients there was a marked increase in the size of the testes, but no signs of increased virilization nor any increase in the urinary 17 ketosteroid excretion, it can be assumed that the secretion of FSH is much greater than that of ICSH. Further evidence supporting this assumption is the histological appearance of these testes which showed a predominance of tubular development, but no increase in the number of Leydig cells. Unfortunately, it was not possible to perform plasma gonadotrophin determinations in the remaining three patients. The absence of an advanced bone age in these boys with precocious puberty may be due not only to the deficiency in androgens, but to the primary deficiency of thyroxine which is necessary for normal bone growth and maturation.

It is noteworthy that De La Balze *et al* (1) reported an enlargement of testes in 3 of 6 patients with hypothyroidism who presented with delayed puberty. In these patients, however, the histological picture was abnormal, showing edema of the interstitial tissue and thus differing from that seen in our patients; furthermore the 3 older patients also had sexual hair and urinary gonadotrophins. However, the observation made by De La Balze *et al* proves that in cases of primary hypothyroidism, the existence of enlarged testes alone should not be taken as evidence for precocious sexual development. The presence of other secondary sexual characteristics, maturation of the seminiferous cells of the testes, as shown by biopsy, and an increase in serum gonadotrophins must all be confirmed before this diagnosis can be made.

There is no data available as to the correlation between the aetiology of hypothyroidism, its duration before institution of treatment and the appearance of precocious signs of sexual development. All of our patients had had varying degrees of hypothyroidism from infancy. Three received no treatment and one probably received inadequate and irregular medication. It is interesting that two of these were brothers and had a metabolic type of hypothyroidism. These patients 3 and 4 had only a slight growth retardation and in patient 4, the skeletal retardation was less than 3 years. This can be assumed from an incomplete enzymatic de

100 ml Thyroid radioactive ¹³¹I uptake was 18° after 2 hours and 38 after 24 hours ¹³¹I chromatography (150 μci) revealed a conversion ratio of 4 5 After 24 hours there was 6 2 of the T3

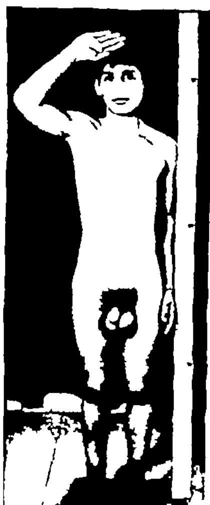


Fig 5 Patient A W at the age of 19 years Note markedly enlarged testicles volume >50 ml

in the plasma This amount disappeared after 4 hours Large amounts of free iodine were found i the urine during 24 hours These findings are co sistent with hypothyroidism due to an enzymatic de fect in the synthesis of thyroxine

Within 2 years of treatment with a thyroid extra or with l thyroxine there was no change in heigh mental state or testicular volume (Fig 5) Testic biopsy performed at the age of 19 years reveale a histological structure compatible with that foun in adulthood

Patient 4 N W a brother of A W was fir examined at the age of 12 /₁₁ years Like h brother this patient had shown signs of motor an mental retardation since early infancy Physical ex amination showed a boy of proportional body buil with a slow sluggish speech His height was 135 cm corresponding to a height age of 10 years Hi bone age was 10 years Axillary hair was absen and only fuzzy hair was found in the pubic are The penis length was 5 3 cm and the testes wer large for his age measuring 15 ml in volume

Laboratory findings were PBI 5 0 μg/100 ml BEI 2 8 μg/100 ml Thyroid radioactive I up take was 21% after 2 hours and 41 after 24 hours ¹³¹I chromatography (100 μci) showed a con version ratio of 10 % with the same percentage o distribution of T and T in the plasma as in h brother (patient 3) Urinary 17 ketosteroids were 3. mg/24 hours Urinary gonadotropins were 4 MUU 24 hours X ray of the skull and the EEG wer normal

The boy received no treatment within the nex year during which time there was a further develop ment in the secondary sexual characteristics and an increase in the size of the testes the volume of which reached 18 ml Even after treatment with l thyroxine was begun the testes continued to increase in size reaching a volume of 25-30 ml but we are

Table 1 *Pertinent data on male patients with hypothyroidism and precocious sexual development*

Authors and references	Age at which onset of hypothyroidism was noted years	Age at first examination years	Plasma PBI μg/100 ml	RAI¹³¹ uptake at 24 hours	TSH reserve test	Pathologic plasma I¹³¹ chromatography	Enlarged testicles	Testicular biopsy	Virilization	Urinary gonadotrophins M U U /24 hrs	Plasma FSH	Plasma LH	Enlarged sella by X ray	Reversal of symptoms on therapy
Franks & Stempfel (4)	5	7ʳ	3 2	4	–		+	P	–	–			–	+
Hubble		25					+		–	–				
Maranon et al (9)ᵃ							+		+					
Present report														
Pt no 1 M Y	2	6ᵃ	1 8	10 5	–		+	P	–	–	↑	↑	+	–
Pt no 2 J S	3	14	2 5	4 5	–		+		–	3			+	+
Pt no 3 A W	1	17	5 8	38		+	+	N	–	6			–	–
Pt no 4 N W	1	12ᵃ	5 0	41		+	+	N	–	4			–	

+ = Positive – = Negative ↑ = Increased N = Normal P = Precocious maturation
ᵃ = A case associated with Down s syndrome

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fect in the thyroid leading to progressing thyroxin insufficiency with increasing age and enlarging body mass To the best of our knowledge this is the first description of this kind of hypothyroidism associated with precocious sexual development

SUMMARY

Four boys with primary hypothyroidism and enlarged testicles are described The patients were studied at the ages of 6 $^{6}/_{12}$ 12 $^{8}/_{12}$ 14 $^{9}/_{12}$ and 17 $^{6}/_{12}$ years Two had hypothyroidism of unknown etiology The other two who were brothers, suffered from an enzymatic error in thyroxine synthesis The common features were precocious and abnormal enlargement of the testicles In the youngest patient precocious maturation of the seminiferous cells was proved by testicular biopsy It is assumed that the aetiology of this syndrome is a hormonal overlap between TSH and gonadotrophins, a hypothesis which is favored by the finding of increased plasma gonadotrophins in the only patient tested The marked enlargement of the testicles without any excessive virilization is assumed to result from a greater secretion of FSH than of ICSH

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REFERENCES

1 Balze De La F A Arrilaga F Mancini R E Janches M Davidson O W & Gurtman A I Male hypogonadism in hypothyroidism *J Clin Endocr 22* 217 1962

2 Bergstrand C G A case of hypothyroidism with signs of precocious sexual development *Acta Endocr 20* 338 1955

3 Franchimont P Personal communication

4 Franks R C & Stempfel R S Juvenile hypothyroidism and precocious testicular maturation *J Clin Endocr 23* 805 1963

5 Hubble D Endocrine relations *Lancet I* 1 1965

6 Kendle F W Case of precocious puberty in a female child *Brit Med J I* 246 1905

7 Lederer J *Les relations thyro-ovariennes* Masson Paris 1946

8 Lederer J Roger F & Bataille J P *Le mechanism de l insuffisance génitale masculine dans l hypothyroidie primaire* Actualites Endocrinologiques (La Pitie) L Expansion Paris 1967 p 231

9 Maranon G Martinez D J & Gochi M J M Mongolism et Macrogenitosomie *Ann Endocr* (Paris) *23* 41 1951

10 Pabst H F Pueschel S & Hillman D A Etiologic interrelationship in Down s syndrome Hypothyroidism and precocious sexual development *Pediatrics 40* 590 1967

11 Tanner J M *Growth at Adolescence* Blackwell Scientific Publ Oxford 1962 2nd ed

12 Vague J & Favier G Les retards pubertaires chez la fille *Rev Prat* (Paris) *44* 109 1958

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ATAXIC SYNDROME IN CONGENITAL HYPOTHYROIDISM

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From the Department of Paediatrics, University Hospital, Uppsala, Sweden

The adverse effect on the developing brain of an intra- or extrauterine athyroid or hypothyroid state is well known. The classical result is mental retardation of a variable degree. Behavioral deviations and abnormalities recorded at electroencephalographic examination (1, 17) have also been noted but neurological syndromes with motor handicaps are only seldom reported.

In a follow-up study comprising all cases of ataxia in infancy and childhood from the Uppsala area during the years 1956–1961, one of the authors (6) had two mildly ataxic patients with congenital hypothyroidism, one of them adequately substituted since the age of 8 weeks. Since this preliminary observation all cases in Uppsala with the diagnosis of congenital hypothyroidism have been particularly observed for the presence or absence of ataxic signs. This paper presents our experiences from a small but unselected clinical series collected during a 12 year period.

CLINICAL MATERIAL

In the area of Uppsala with about 180 000 inhabitants 11 children with congenital hypothyroidism were admitted in 1956–1969. In addition one child (S. L.) referred to the hospital from another region. Data about these patients are summarized in Table 1. The treatment was started as soon as the diagnosis was proved, either desiccated thyroid or sodium thyroxine being used. In all cases the dose was kept at the highest tolerated. The treatment was considered sufficient when evidence of normal skeletal maturation, normal growth, development and normal PBI and cholesterol was observed. Physical, motor development and neurological examinations were performed regularly.

RESULTS

The results of these examinations are summarized in Table 1. Ataxia of the cerebellar type was found in 6 of the 12 patients and three of them are described in some detail below.

Case 1

T. B., record no UAS 303/61. This boy, born February 12, 1955, is the eldest child of three in a healthy family. The delivery was uneventful 3 weeks before term and the birth weight 3470 g. Because of vomiting and moderate neonatal icterus (upper level at 16.0 mg/100 ml on the 11th day) he was seen at the pediatric clinic during the neonatal period but no abnormal neurological signs were observed. It was 6 weeks before his icterus had completely disappeared.

When 10 ½ months old he was again sent for consultation because of late psychomotor development (not able to sit or creep) and a myxedematous appearance. PBI was 1.6 microgram/100 ml and cholesterol 250 mg/100 ml. The neurological findings were considered normal except for a convergent squint. He was put on a relatively high dose of thyroid which he has since been receiving in a well controlled dosage.

He walked when 25 months old but was early considered to be a very clumsy child, infantile for his age and retarded in his speech development. When re-examined neurologically at 4 ½ years of age his way of running was found to be abnormal and dysharmonic, and he had a slight but evident cerebellar ataxia with intention tremor and retarded speech.

When 6 years old he was observed for 1 week at

fect in the thyroid leading to progressing thyroxin insufficiency with increasing age and enlarging body mass To the best of our knowledge this is the first description of this kind of hypothyroidism associated with precocious sexual development

SUMMARY

Four boys with primary hypothyroidism and enlarged testicles are described The patients were studied at the ages of $6\,^{9}/_{1}$ $12\,^{8}/_{1}$ $14\,^{9}/_{1}$ and $17\,^{5}/_{1}$ years Two had hypothyroidism of unknown etiology The other two who were brothers suffered from an enzymatic error in thyroxine synthesis The common features were precocious and abnormal enlargement of the testicles In the youngest patient precocious maturation of the seminiferous cells was proved by testicular biopsy It is assumed that the aetiology of this syndrome is a hormonal overlap between TSH and gonadotrophins, a hypothesis which is favored by the finding of increased plasma gonadotrophins in the only patient tested The marked enlargement of the testicles without any excessive virilization is assumed to result from a greater secretion of FSH than of ICSH

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The results of these examinations are summarized in Table 1 Ataxia of the cerebellar type was found in 6 of the 12 patients and three of them are described in some detail below

Case 1

T B record no UAS 105/61 This boy born February 12 1955 is the eldest child of three in a healthy family The delivery was uneventful 2 weeks before term and the birth weight 3470 g Because of vomiting and moderate neonatal icterus (upper level at 16 0 mg/100 ml on the 11th day) he was seen at the pediatric clinic during the neonatal period but no abnormal neurological signs were observed It was *6 weeks before his icterus had completely disappeared*

When 10 ½ months old he was again sent for consultation because of late psychomotor development (not able to sit or creep) and a myxedematous appearance PBI was 1 6 microgram/100 ml and cholesterol 250 mg/100 ml The neurological findings were considered normal except for a convergent squint *He* was put on a relatively high dose of thyroid which he has since been receiving *in a well* controlled dosage

He walked when 25 months old but was early considered to be a very clumsy child, infantile for his age and retarded in his speech development When re-examined neurologically at 4 ½ years of age his way of running was found to be abnormal and dysharmonic and he had a slight but evident cerebellar ataxia with intention tremor and retarded speech

When 6 years old he was observed for 1 week

Table 1 *Clinical material of congenital hypothyroidism admitted 1956–1969*

Case	Date of birth	Sex	Hypothyr diagnosed age (months)	PBI (µg/100 ml)	Walked age (months)	Ataxia diagn age (years)	Intellectual level	Latest follow up age (years)	Running	Alternating jump	Intention tremor	Writing style	Pinpoint test
T B	550212	♂	11	—	25	4½	IQ 50–60	14	Dysharmonic	Dysharmonic	+	Bad	Tremor
M A	640815	♀	24	0 8	31	2½	IQ 80	5	Dysharmonic	Impossible	+ +	—	Tremor
S L	620620	♀	9	1 8	—	3	Imbecile	7	—	—	+ + +	—	—
L L	511019	♂	10	—	25	8	IQ 64	16	Clumsy	Impossible	+ + +	Bad	Tremor
M K	570314		15	1 4	24	4½	Low normal	12	Normal	Normal	+	Good	?
A S	580309	♀	2	2 5	19	3	IQ 94	11	Dysharmonic	Dysharmonic	—	Normal	Normal
T N	550530	♂	6	—	16	—	Normal	6	Normal	Normal	—	—	—
L K	560406	♂	3	1 7	19	—	IQ 63	13	Normal	Normal	—	Good	—
I B	601229	♂	6	2 5	25	—	Imbecile	7	Clumsy	—	—	Normal	Normal
E B	621016	♂	10	2 2	16	—	Low/educable	5	Normal	—	—	—	—
N W	630305	♂	1½	0 8	16	—	Normal	6	Normal	Normal	—	—	Normal
H G	660423	♂	16	2 4	14	—	Normal	4	Normal	Normal for age	—	—	—

the pediatric clinic He was found to be mentally retarded with an intelligence quotient of 48 according to Terman Merill and 58 according to Buhler Hetzer His ataxic signs of the cerebellar type were once again established EEG was *abnormal* with numerous epileptogenic discharges but he had no convulsions. He was sent to a school for mentally retarded and has been there ever since

In spite of intensive training during the following years he still had great difficulties in learning common motor performances Thus he was 1? years of *age when he was first able to* ride a two wheel bicycle after about 4 years of training and was still unable to skate at the age of 15 years His fine motor dysfunction was less remarkable but was obvious in daily life and in his style of writing

When last seen at 14 years of age he had obvious difficulties in buttoning his clothes tying his shoe *laces and filling a glass without spilling His* gross motor function was fairly well compensated but his running was stiffer and slower than normal Neurological examination still revealed a relatively well compensated cerebellar ataxia with intention tremor but no hyperkinesia and no signs of spasticity In summary his ataxic abnormality had gradually become less evident in his daily life during the last few years

Case 2

M A record no UAS 166/66 The first child of two in a healthy family this girl was born on August 15 1964 3 weeks before term There were no complications during the pregnancy or delivery and the birth weight was 3200 g Because of suspected dislocation *of the hip the child was seen at the hospital* at the age of 6 weeks The hips were found to be normal but the child had a prolonged neonatal persistent icterus (bilirubin 7 2 mg/ 100 ml) Physical and neurological examination showed nothing abnormal A blood sample for PBI was taken but for some reason was not analyzed The family then *moved to another part of the country*

It soon became obvious that the child s psychomotor development was retarded (she was able to sit at the age of 11 months and to creep at 1? months but was not able to walk at 24 months) At the age of 24 months the child was admitted to a pediatric clinic She exhibited a myxedematous appearance and *her growth was considerably stunted* The bone age was considered to be about 8 months and the PBI was 0 8 microgram/100 ml On motor age examination she showed a developmental level of about 11 months and muscular hypotonia particularly in the lower limbs However no other neurological signs were noted She was put on a fairly high dose of thyroid and since then the dosage has been kept at the upper tolerated limit

She learned to walk at the age of 30 months but was found to be very clumsy On re examination when 3 years old an evident cerebellar ataxia with intention tremor and a very dysharmonic way of

running were noted Her speech was markedly retarded Intensive motor training was started

The girl was last seen in Uppsala at the age of 4 / years and the cerebellar type ataxia was still evident Her intention tremor was very obvious She was unable to fill a glass without spilling or to button her clothes Her running was clumsy and slow and she was not able to jump in an adequate way for her age No spastic signs were noted She was found to have an intelligence quotient of 80 according to Terman Merrill

To summarize this girl has a marked cerebellar ataxia now giving more problems in daily life than her moderate and uncomplicated mental retardation

Case 3

S L record no UAS 1949/66 This girl born in June 20 1962 is the second of three children Her parents and siblings are all healthy Pregnancy and delivery were uneventful Her birth weight was 3770 g She was said to be slightly icteric already at birth Her icterus was never severe and it disappeared after one week Difficulties with feeding and general dullness were already observed during the neonatal period Hypothyroidism was diagnosed at her pediatric county clinic when she was 9 months old mainly on the basis of her slow psychomotor development Her PBI was found to be 1 8 microgram/100 ml Substitutional therapy was immediately instituted

The girl learned to roll over when 12 months old and to sit without support at 24 months She was still markedly unsteady and swaying when first seen in Uppsala at 3 / years of age She was also able then to shuffle along on her buttocks Her motor age was found to be about 8 months at that stage An obvious ataxia with slight athetoid movements of the hands was observed

When 4 years old she was seen for neurological consultation She was still unable to walk and her motor age was found to be 10 months in the arms and hands and 8 months in the lower extremities Neurological examination revealed a pronounced truncal ataxia with coarse truncal tremor and intention tremor in the arms and hands In addition she had slight hyperkinetic movements of the athetoid type There were no signs of spasticity Her intellectual capacity was reduced No formal testing was possible but it was judged that she functioned at an imbecile level EEG was within normal limits on two different occasions Air encephalography showed a moderate atrophic encephalopathy with generalized dilatation of the ventricular system but no evidence of cortical atrophy

At 7 years of age she was a little more steady in the sitting position but was unable to stand or walk She could not eat nor dress herself and had no speech Otherwise the condition was unchanged in all essential respects She was now being cared for in a county home for imbeciles and was considered to function at a relatively high imbecile level

DISCUSSION

Half of our 12 cases had ataxic signs of the cerebellar type with incoordination of movements and intention tremor but no objective evidence of components of dysequilibrium In no case were signs of spasticity observed The ataxia was nonprogressive rather showing a tendency towards compensation and functional improvement Four children were only slightly affected and their condition might well have been overlooked had not a thorough history been taken and repeated neurological examinations performed Their clumsiness and tendency to spill their drinks had on many occasions been attributed erroneously to mental dullness The ataxia in one child was moderate in degree and one other child had a very pronounced and disabling motor handicap which in fact brought her to the clinic for neurological examination independent of her congenital hypothyroidism It can always be argued that in this case the syndrome of cerebral palsy may be due to quite a different unknown prenatal lesion or malformation parallel to the congenital hypothyroidism However in view of the similarity to the other five of our children this possibility seems fairly unlikely

In older literature no ataxic but some diplegic syndromes have been reported in association with congenital hypothyroidism e g by McGarrison (14) and Naville (15) but their clinical description is consistent with the concept of spastic diplegia and no cerebellar signs seem to have been recorded

In recent years some interesting observations concerning this subject have been made In a review concerning the mental prognosis in 128 cases with hypothyroidism of infancy and childhood Smith and collaborators (18) found neurological signs such as shuffling gait incoordination awkwardness coarse tremor and spasticity in 26 of 79 patients with severe congenital hypothyroidism No such signs were found in 32 cases with mild hypothyroidism Another interesting point was that abnormal neurologic findings were noted in only 4 of 22

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ACKNOWLEDGEMENT

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REFERENCES

1 d'Avignon M & Melin K A The electroencephalogram in congenital hypothyreosis *Acta Paediat Scand 38* 37 1949
2 Eayrs J T The vascularity of the cerebral cortex in normal and cretinous rats *J Anat 88* 164 1954
3 — The influence of the thyroid on the central nervous system *Brit Med Bull 16* 122 1960
4 Eayrs J T & Taylor S H The effect of thyroid deficiency induced by methylthiouracil on the maturation of the central nervous system *J Anat 85* 350 1951
5 Geloso J P Hemon P Legrand J Legrand C & Jost A Some aspects of thyroid physiology during the perinatal period *Gen Comp Endocr 10* 191 1965
6 Hagberg B Ataxia in children 1 Survey and 5 years clinical material *Lakartidningen 59* 1530 1962
7 Jellinek E H & Kelly R E Cerebellar syndrome in myxoedema *Lancet II* 225 1960
8 Klosovskij B N *The development of the brain and its disturbance by harmful factors* Pergamon Press Oxford London New York and Paris 1963 pp 152–161
9 Legrand J Endocrinologie—Influence de l'hypothyroidisme sur la maturation du cortex cerebelleux *C R Acad Sci 261* 544 1965
10 — Analyse de l'action morphogenetique des hormones thyroidiennes sur le cervelet du jeune rat *Arch Anat Micr Morph Exp 56* 206 1967
11 — Variations en fonction de l'age de la response du cervelet a l'action morphogenetique de la thyroide chez le rat *Arch Anat Micr Morph Exp 56* 291 1967
12 Legrand J Kriegel A & Jost A Deficience thyroidienne et maturation du cervelet chez le rat blanc *Arch Anat Micr Morph Exp 50* 507 1961
13 Lotmar F Entwicklungsstorungen in der Kleinhirnrinde beim endemischen Cretinismus *Z Ges Neurol Psychiat 136* 412 1931
14 McGarrison R *The thyroid gland in health and disease* Wm Wood & Co Baltimore 1917 p 141
15 Naville F Congenital diplegia and thyroid disturbance in abnormal infants of Geneva *Schweiz Arch Neurol Neurochir Psychiat 13* 559 1923
16 Price T R & Netsky M G Myxedema and ataxia Cerebellar alterations and "neural myxedema bodies" *Neurology 16* 957 1966
17 Schultz M A Schulte F J & Parmelee A H Schilddrusenhormon und Hirnentwicklung *Msche Kinderheilk 115* 284 1967
18 Smith D W Blizzard R M & Wilkins L The mental prognosis in hypothyroidism of infancy and childhood *Pediatrics 19* 1011 1957

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Key words Ataxia congenital hypothyroidism

patients treated within the first 6 months of life whereas 22 of 57 patients treated after 6 months of age showed such signs Furthermore the degree of mental retardation was propor tionate to the degree of neurologic impairment In Fumer's textbook *Pediatric Neurology*, Jenkins in 1964 made the following statement spasticity tremor extrapyramidal rigidity choreiform and athetotic movements, and optic nerve involvement may be seen Klosovskij (8) mentions a group of 12 children, aged 2–11 years insufficiently substituted with thyroid hormone all twelve exhibited tremor of all ex tremities and three of them showed increased tendon reflexes and pyramidal signs

Experimental studies on animals have con firmed the clinical and neuropathological evi- dence of the delay or arrest of brain develop ment in man Eayrs and his group (3 4) obser ved several morphogenetic changes of the cer ebral cortex in rat litters following artificially induced hypothyroidism at birth Legrand & Jost and their collaborators (5 9 10 11 12) have made extensive corresponding studies on cerebellar cortex They found that the histolog ical maturation of the whole cerebellar cortex was altered and delayed in rats made hypo thyroid through treatment with radioactive io- dine and propylthiouracil before at or shortly after birth (within the first two weeks) How ever thyroidectomy was found ineffective when performed after postnatal day 14 It ap- pears that cerebellar structures seem to be par ticularly dependent upon an adequate action of thyroid hormone during intrauterine perinatal and early postnatal life With all probability corresponding time schedules should also be valid on the whole for man the length of the postnatal risk period being unknown however It is logical to believe that this period rather might tally in months than in weeks and this is supported by the findings of Smith and col laborators (18)

It is of therapeutic interest to emphasize that thyroxin treatment when initiated before the end of the second postnatal week and not continued after that period was able to correct all these alterations in the cerebellum of young rats This constitutes yet further support for a particular and time limited stage of sensitivity to the hormone

Interesting neuropathological observations supporting the validity of the animal studies also in man were made in adult cretin patients at an early date by Lotmar (13) Among other things, he found localized islands of fetal cere bellar cortex mixed in corresponding tissue of adult structure He also revealed Purkinje cell changes, well in agreement with a cellular mat urational arrest as found in the previously mentioned animal studies by Legrand *et al* (12)

To summarize both experimental and neu ropathological findings have provided a plausi ble explanation for the origin of ataxic syn dromes in infants and children with congenital hypothyroidism not treated during their first months of life From our cases it is of interest to note that case 6 was rather hyper than hyposubstituted with thyroid hormones from as early an age as 8 weeks and yet developed a mild but quite obvious ataxic syndrome Thus the time limit for full prevention of cerebellar maturational derangement may be short also in the postnatal life of man

SUMMARY

Ataxia of the cerebellar type was found in six of twelve patients with congenital hypothyroid ism admitted to the department of pediatrics of Uppsala during the years 1956–69 The me dian age for diagnosis and the start of adequate substitution therapy was 9–10 months the youngest patient being 6 weeks and the eldest 24 months The ataxia was non progressive with a tendency towards compensation and functional improvement Four cases were only slightly one moderately and one severely disa bled Animal experiments and neuropatholog ical observations in man supporting a cellular maturational arrest of cerebellar structures in congenital hypothyroid states are discussed

Pat	J B	T J	M J															
Age	0.4	0.4	0.5	0.9	0.9	3.1	4.8	[illegible]										
Sex	M	M	M	M	M	M	M	F	M	F	F	F						
General symptoms																		
Headache								+	+		+	+		+	+	+	+	(+)
Nausea and vomiting					+		+	+		+	+	+	+			+	+	
Dizziness											+	+					+	
Tinnitus												+						
Local signs																		
Bulging fontanel		+		+														
Suture diastasis		+	+	+	+		+											
Ophthalmological																		
Papilledema						+	+	+	+		+	+		+	+	+		+
Unsharp disc edges										+			+				+	
Blurring of vision							+	+		+		+		+		+		
Diplopia												+	+					
Squint VIth nerve palsy						+	+	+	+	+		+				+		
Nystagmus								+		+			+					
Visual field defects												+		+		+		
Visual hallucinations								+										
Mental signs																		
Irritability				+			+	+		+		+						
Nervousness										+		+					+	
Disturbed memory									+	+		+						
Apathy		+	+	+	+		+					+			+			
Disturbed ability to concentrate								+		+		+						
Other neurological signs																		
Facial weakness												+						
Facial numbness												+	+					
Pyramidal signs													+					
Cutaneous sensations													+					
Investigations																		
CSF pressure				H							H							
CSF protein	N		N	N	N		N	N	N	N	N	N	N		N			N
CSF cells	N		N	N	N		N	N	N	N	N	N	N		N			N
Skull X ray					P	N	P	N	N	N		N	N	N	N	N	N	N
EEG						N	P	N	N	P		P	P	P	N	P		N
Carotid angiography								N	N				N		N			N
Air ventriculography						N	N	N		N		N	N					N
Isotope encephalography														N				N
Echo encephalography					N		N											

+ = positive, M = male, F = female, H = high, P = pathological, N = normal.

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REVIEW ARTICLE

BENIGN INTRACRANIAL HYPERTENSION (PSEUDOTUMOR CEREBRI)

Review and Report of 18 Cases

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Benign intracranial hypertension is one of several names given to a clinical syndrome of prolonged increased intracranial pressure, being due neither to a localized space process nor to the acute brain swelling effects of meningitis or encephalitis It occurs in association with various etiologically known disorders such as sinus thrombosis and in some less well defined conditions such as para infectious and allergic states There is also an idiopathic form (7 38) Synonymous names from the literature are pseudotumor cerebri brain swelling of unknown cause hypertensive meningeal hydrops otitic hydrocephalus and toxic hydrocephalus The condition was first described by Quincke in 1893 (62) under the designation of serous meningitis Nonne introduced the name pseudotumor cerebri in 1914 (55) Davidoff & Dyke in 1936 (13) demonstrated that this syndrome occurred in the presence of a normal ventricular system In most cases air encephalography or angiography must be performed in order to exclude the possibility of brain tumor These patients usually complain of headache, vomiting blurring of vision and diplopia In cases with closed cranial sutures, papilledema is always noted and VIth nerve palsy and disturbed balance are sometimes observed Focal neurologic signs are only seldom encountered In infants a bulging fontanel and diastasis of sutures may be the only signs Lumbar or ventricular puncture reveals a normal composition of the cerebrospinal fluid (CSF) but an increased CSF pressure This does not often affect the general condition, which is good in proportion to the magnitude of the signs of increased intracranial pressure

As cases with the syndrome of pseudotumor cerebri often constitute large clinical difficulties for differential diagnosis and as our knowledge of the etiology and pathogenetic mechanisms of this condition are still incomplete we considered it worthwhile to summarize our own clinical experiences from 18 children, to give some characteristic case reports and to review the relevant literature

OWN CLINICAL MATERIAL

During the years 1960–69 18 infants and children at our clinics were diagnosed as having pseudotumor cerebri This series is summarized in Table 1 The average age of the patients was 6 years and 7 months a variation between 4 months and 19 years There were 10 boys and 8 girls Sixteen of the cases were diagnosed and treated in inpatient clinics and two at outpatient clinics Some representative cases will be presented below

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In 1965 two years after the onset of her disease
she had no headache and had passed her 4th school
year without difficulties Her temperament was said
o be more labile than before the disease Her VIth
and VIIth nerve palsies had completely disappeared
The vision was 0 7 on the right eye and 0 3 on the
left The discs were abnormally pale but had sharp
edges and no protrusion EEG had further improved
but still showed a slow background activity When
last contacted for follow up in April 1969—6 years
after her dramatic illness—she was still in the same
good condition and had no difficulties in carrying
out her school work

Comments This is a purely idiopathic case and
a severe one Of interest are the recurrent episodes
of headache and vomiting occurring during the 3
years preceding her real illness Histories of this type
are not seldom found in the literature concerning
purely idiopathic cases

ETIOLOGY

Being a diagnosis of exclusion several different explanations have been presented for pseudotumor cerebri and its etiology (Table 2)

One of the *endocrine* factors of main importance is undoubtedly a prolonged steroid therapy state which occurs in connection with the pseudotumor syndrome practically only in children (17 75) Any corticosteroid or ACTH preparation may be involved but triamcinolone has been reported surprisingly often (17) Many different diseases treated with steroids or ACTH and complicated by abnormally raised intracranial pressure have been reported (8 9 14 27 33 45 73 75) Children receiving long term steroids for asthma rheumatoid arthritis or blood dyscrasias are characteristic victims (78) Symptoms of pseudotumor have most often occurred during rapid reduction or withdrawal of the corticosteroid regimen (33 66)

Table 2 *Different abnormal conditions associated with pseudotumor cerebri*

1 Endocrine conditions
- Hypoadrenalism (14 4? 75)
- Hypothyroidism (59)
- Hypoparathyroidism (1 16 71)
- Pseudohypoparathyroidism (16)
- Hyperadrenalism (31)
- Hyperthyroidism (29)
- Ovarian dysfunction (34 35 51)
- Oral contraceptive use (77)
- Corticosteroid patients (14 17 30 33 66 75)

2 Metabolic dysfunction
- Vitamin A deficiency (2, 10)
- Vitamin A overdosage (48 61)
- Vitamin B deficiency (44)
- Uremia pseudouremia (44)
- Hypophosphatasia (25 43)
- Obesity (6 36 60)

3 Non-endocrine or non metabolic systemic diseases
- Anemias of different types (37 40 47 65 69)
- Polycythemia vera (46)
- Leukemia (21)
- Aldrich s syndrome (37)
- Chronic respiratory insufficiency (45)
- Alkalosis (78)
- Congenital or acquired heart disease (45 81)

4 Allergic conditions
- Food allergy (45)
- Drug allergy (4 45 53 68 76)
- Allergic diseases (15)

5 Non-cerebral infections
- Otitis media (19 50 52, 72)
- Paranasal sinusitis (19 50 52, 72)
- Ethmoiditis (19 50 52 72)
- Poliomyelitis (28 54)
- Roseola infantum (58)
- Guillain Barré s syndrome (24)

6 Other conditions
- Lymphogenous encephalopathy (?)
- Skull trauma (3 6 23)

7 Idiopathic states (39)

In adulthood one peculiarity is that 2/3–3/4 of the patients are women (6 22 74) particularly obese ones An abnormal estrogen metabolism has therefore been implicated owing to the common findings of association with menstrual edema premenstrual tension menstrual irregularities menarche menopause pregnancy miscarriage or puerperal periods However no objective support from endocrinological studies has been obtained (57 78) Such studies speak more in favour of a defect in the pituitary-corticotropin adrenal axis (57)

The connection between pseudotumor cerebri and obesity is still more obscure but is

Case 1

M K This girl was born June 4 1951 as the first child in a healthy family Her earlier years had been uneventful except for an alternating squint diagnosed when she was 3 years old Because of headache when reading she was given glasses in 1962 and markedly improved

During the autumn 1963 she developed 1-3 times per week a new form of headache particularly starting early in the morning The headache was localized diffusely to both sides of the forehead and the parietal regions Sometimes it was associated with nausea After about one year a marked deterioration occurred The headache appeared every morning and she felt dizzy and sick but never vomited This deterioration coincided with her menarche which appeared August 1963 and during the following months her symptoms were exaggerated during the menstrual period

In October 1964 when 13 / years old she was examined at the pediatric department in the county hospital She was found to be large and well developed for her age General and neurological examination showed nothing abnormal except for the eye findings She still had an alternating squint Her vision was 0 3 on the right side 0 9 on the left Both discs were edematous and protruding about 3 D Skull X ray was normal EEG showed slight suppression of the basal activity but no focal abnormalities Otoneurological findings were normal

The girl was sent to the University Hospital of Uppsala with a suspected intracranial tumor Neuroradiological examinations were planned but were postponed owing to gradual improvement About 1 month later her vision was found to be 1 0 bilaterally and the disc protrusion had diminished In December 1964 the discs were quite normal

When last seen in July 1968 at her 5 year follow up examination this girl still complained of headache during certain periods but was otherwise healthy Her vision was found to be 0 9 bilaterally and the ocular fundi were normal Nothing abnormal was found on neurological examination

Comments This teenage girl is one among the known cases where a pseudotumorous state appears in a girl during puberty and with a seemingly close connection between exaggeration of the symptoms and her menstrual period It would seem highly probable that endocrine factors might have been of importance for the development of the syndrome even if the mechanism remains quite obscure

Case 2

R B A boy 4 / years old born February 3 1963 previously healthy 4th child in a healthy family He was treated at the ENT department University Hospital of Uppsala October 9-13 1967 with penicillin for a left sided peritonsillitis but was sent home afebrile and in a good condition One day later he became tired and apathetic and slept almost the whole day During the next few days he vomited several times gradually started to squint and developed a slight ptosis on the right side

When examined at the pediatric department on October 17 1967 he shifted between a state of apathy and one of hyperirritability He was slightly ataxic and could not walk without support He had a left VIth nerve palsy protruding discs (2-3 D) and peripapillary streaks of hemorrhage EEG was diffusely abnormal without any normal background activity and showed irregular delta activity particularly on the right occipital region X ray revealed widened sutures *Echo-encephalography* gave normal findings with an ordinary midline echo An intracranial abscess was suspected and he was put on high doses of *penicillin*

The boy was transferred to the neurosurgical department where lumbar encephalography and ventriculography were performed These examinations revealed nothing abnormal except for marked difficulties in getting air to the convexity of the brain well consistent with a brain edema

During the next few weeks the boy gradually improved but one month after appearance of the first symptoms he still had a disc protrusion of 2 D a left sided VIth nerve palsy and slight ataxia During the next year his ataxia gradually disappeared but the other signs remained unchanged

When last seen in July 1969 he was in a very good general condition neurologically normal and now without any sign of VIth nerve palsy His discs were still blurred but only slightly protruding and his vision was quite normal

Comments This 4 / year-old boy gives an example of a pseudotumorous state probably due to parainfectious mechanisms

Case 3

A M This girl born October 6 1956 was the third of four children in a healthy family Her earlier psychomotor development had been quite normal and she passed her first school year with good marks During the years 1960–63 she had periods of headache and vomiting lasting 1-2 days with free intervals of some months She was examined by a pediatrician in April 1963 but nothing abnormal was revealed

On the 28th of July 1963 when 7¹/ years old she again had an acute attack of headache mainly right frontal and in addition vomiting lasting about 24 hours After two days she became dizzy had double vision and developed an unsteady gait Because of increasing apathy and tiredness she was sent to the pediatric department of the county hospital On examination she was found to have bilateral VIth nerve and right VIIth nerve palsy anisocoria no corneal reflex on the left side and papilledema with a disc protrusion of 2-3 D bilaterally She was sent to the University Hospital of Uppsala on *the 4th of* August suspected of having a brain tumor Air encephalography since burr holes had been made revealed a normal ventricular system with no dislocation The CSF was normal EEG was severely ab

-ground with no normal background activity and diffuse symmetrical paroxysmal episodes. During the next month she deteriorated in spite of steroid treatment with 8 mg Dexamethason® per day.

Because of increasing pain, rapidly decreasing visual acuity nearly to blindness, disc protrusions of 5 D and abundant leakage of CSF through the burr holes, bilateral subtemporal decompression was performed in September. Her general condition and vision thereafter improved. One month later her vision was 0.2 on the right eye and finger counts on the left. During the following month further improvements in vision and EEG were recorded.

In 1965, two years after the onset of her disease, she had no headache and had passed her 4th school year without difficulties. Her temperament was said to be more labile than before the disease. Her VIth and VIIth nerve palsies had completely disappeared. The vision was 0.7 on the right eye and 0.3 on the left. The discs were abnormally pale but had sharp edges and no protrusion. EEG had further improved but still showed a slow background activity. When last contacted for follow up in April 1969—6 years after her dramatic illness—she was still in the same good condition and had no difficulties in carrying out her school work.

Comments: This is a purely idiopathic case and a severe one. Of interest are the recurrent episodes of headache and vomiting occurring during the 3 years preceding her real illness. Histories of this type are not seldom found in the literature concerning purely idiopathic cases.

ETIOLOGY

Being a diagnosis of exclusion, several different explanations have been presented for pseudotumor cerebri and its etiology (Table 2).

One of the *endocrine* factors of main importance is undoubtedly a prolonged steroid therapy state, which occurs in connection with the pseudotumor syndrome practically only in children (17, 75). Any corticosteroid or ACTH preparation may be involved, but triamcinolone has been reported surprisingly often (17). Many different diseases treated with steroids or ACTH and complicated by abnormally raised intracranial pressure have been reported (8, 9, 14, 27, 33, 45, 73, 75). Children receiving long term steroids for asthma, rheumatoid arthritis or blood dyscrasias are characteristic victims (78). Symptoms of pseudotumor have most often occurred during rapid reduction or withdrawal of the corticosteroid regimen (33, 66).

Table 2. *Different abnormal conditions associated with pseudotumor cerebri*

1. Endocrine conditions
   - Hypoadrenalism (14, 42, 75)
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   - Hypoparathyroidism (1, 16, 71)
   - Pseudohypoparathyroidism (16)
   - Hyperadrenalism (31)
   - Hyperthyroidism (29)
   - Ovarian dysfunction (34, 35, 51)
   - Oral contraceptive use (77)
   - Corticosteroid patients (14, 17, 30, 33, 66, 75)
2. Metabolic dysfunction
   - Vitamin A deficiency (2, 10)
   - Vitamin A overdosage (48, 61)
   - Vitamin B deficiency (44)
   - Uremia, pseudouremia (44)
   - Hypophosphatasia (25, 43)
   - Obesity (6, 34, 60)
3. Non-endocrine or non-metabolic systemic diseases
   - Anemias of different types (37, 40, 47, 65, 69)
   - Polycythemia vera (46)
   - Leukemia (21)
   - Aldrich's syndrome (37)
   - Chronic respiratory insufficiency (45)
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   - Congenital or acquired heart disease (45, 81)
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   - Food allergy (45)
   - Drug allergy (4, 45, 53, 68, 76)
   - Allergic diseases (15)
5. Non-cerebral infections
   - Otitis media (19, 50, 52, 72)
   - Paranasal sinusitis (19, 50, 52, 72)
   - Ethmoiditis (19, 50, 52, 72)
   - Poliomyelitis (28, 54)
   - Roseola infantum (58)
   - Guillain Barré's syndrome (24)
6. Other conditions
   - Lymphogenous encephalopathy (26)
   - Skull trauma (3, 6, 23)
7. Idiopathic states (38)

In adulthood, one peculiarity is that 2/3–3/4 of the patients are women (6, 22, 74), particularly obese ones. An abnormal estrogen metabolism has therefore been implicated owing to the common findings of association with menstrual edema, premenstrual tension, menstrual irregularities, menarche, menopause, pregnancy, miscarriage or puerperal periods. However, no objective support from endocrinological studies has been obtained (57, 78). Such studies speak more in favour of a defect in the pituitary-corticotropin adrenal axis (57).

The connection between pseudotumor cerebri and obesity is still more obscure but is

repeatedly reported (12, 22 36 41 60) A rapid gain in weight was closely related to the onset of the raised intracranial pressure in six patients reported by Bradshaw (6)

Vitamin deficiency or overdosage states *have occurred mainly in infants and often in* connection with other notable symptoms and signs Thus, deficiency of vitamin A (2, 10) and vitamin B in pellagra (44) have been present in pseudotumor cases The same also applies to acute (48) and chronic (61) vitamin A intoxication, the latter being of particular diagnostic interest in infancy where the characteristic symptomatology of older children is lacking

Anemia due to iron deficiency (40 47) vitamin B_{12} deficiency (65) hemolytic conditions (37) or blood loss (69) has been suggested as an etiologic factor but *even true polycythemia* has been considered to be the cause (46) The same also applies to other hematological disorders, among others leukemic conditions (21) and Aldrich s syndrome (37)

Many states leading to chronic hypoxia e g chronic respiratory insufficiency congenital (45) or acquired (81) heart disease, have been blamed as a cause of pseudotumor

Allergy in its various forms has been regarded as a possible contributory factor in the etiology (15) but its suggested mechanisms have permitted no critical evaluation (45) Recently a patient with a delayed penicillin reaction considered to be due to increased intracranial pressure was presented (68) The authors suggested this etiology because of the close association with a serum sickness like reaction, the marked worsening of the patient s condition following a benzathine procaine penicillin G injection and the strikingly positive reaction to a penicilloyl polylysine skin test

Tetracyclines (20 56) and nalidixic acid (4 53 76) are the most common drugs associated with benign intracranial hypertension but their possible allergic background has not yet been proved

Previous *infections* (excluding the acute stages of encephalitis and meningitis) have often been said to be responsible for increased intracranial pressure in children The most important are otitis media and paranasal sinusitis (19 52 72) but other infections in the upper respiratory tract and elsewhere in the body may also be the cause (13 22) Pseudotumorous states usually develop days or weeks after the acute stage usually after a free interval or in the later convalescent stage as in poliomyelitis (32 54)

Table 3 *Probable etiology in 18 cases of pseudotumor cerebri*

	Infants	Children	Total
Parainfectious state (upper resp tract)	1	3	4
Reduction of steroid doses	3		3
Ovarian dysfunction in menarche		2	2
Obesity of a marked degree		2	2
Skull trauma Posttraumatic state (?)		1	1
Tetracycline	1		1
Anemia (autoimmune hemolytic)		1	1
Unknown		4	4
	5	13	18

A minimal to moderate *skull trauma* with or without a shortterm loss of consciousness (66) or even with skull fracture (6) has given rise to a clinical picture of pseudotumor Because the head injury may have been rather trivial and the free interval between the injury and clinical symptoms and signs may vary from some days to 18 months (3) it may be difficult to associate these two conditions causatively with confidence

Finally there are also some cases often older children, with no apparent clinical association so called purely *idiopathic* cases Such cases comprised 44 of the pediatric series of Rose & Matson (66) and are even more common in older ages (38)

In our material, a simple infection of the upper respiratory tract including the ears was considered to be the most common etiology (Table 3) which is well in agreement with the pediatric series of cases reported by Rose & Matson (66) A history of reduced dosage of

Table 4 *Suggested pathogenetic mechanisms in pseudotumor cerebri*

I	Cerebral edema
	1 Hypoxia
	2 Electrolyte imbalance
	3 Allergy
	4 Toxic mechanisms
	5 Pituitary ACTH adrenal axis insufficiency
	6 Other endocrine mechanisms
II	Interference with absorption of CSF
	1 Obstruction of dural venous outflow
	2 Spinal protein blockage
	3 Obstruction of cerebral lymph flow

steroids was obtained in three infants All of these three patients had a pure red cell anemia but the hemoglobin was no longer at the bottom level when the onset of brain symptoms occurred Ovarian dysfunction associated with menorrheal troubles was regarded as probably responsible in two cases Obesity of a marked degree was present in connection with a pseudotumorous state in one boy 19 years of age who weighed 110 kg and had pronounced striae all over the body The patient was suspected of having a pituitary tumor but extensive neuroradiological and neuroendocrinological investigations with a follow up of 1 1/ years demonstrated no evidence of a tumor Another obese boy was also found to have normal 17 keto-steroids and 17 hydroxysteroids in the urine

PATHOGENESIS

Cerebral edema has most often been suggested to be the common factor of the pseudotumorous state (Table 4) The edema is probably subtile (78) and different (66) from the usual type which is present, among other conditions in acute toxic encephalopathies (18) and is accompanied by a typical lowered level of consciousness not characterizing pseudotumor (6 13 66 80) Goebell (30) has tabulated 6 different supporting points that speak for cerebral edema as the pathogenetic mechanism in this condition As the prognosis for complete recovery in pseudotumor is good (66) only occasional autopsies have been reported (5 9 42) They have consistently revealed cerebral edema as the main feature In addition Sahs & Joynt (67) in their biopsy material from 10 patients in decompressing operations have demonstrated both extracellular and intracellular edema in brain tissue

Cerebral edema may develop in many ways The blood brain barrier and the membrane function of the brain cells may be damaged by hypoxia in anemias of different types in congenital and acquired heart disease in chronic pulmonary insufficiency and in poliomyelitis with respiratory palsy Electrolyte imbalance has been considered responsible in hypocalcemic tetany in hypoparathyroidism where differences in calcium concentrations extra and intracellularly might cause accumulation of fluid in the cells Steroid and ACTH therapy and hyperadrenalism may in some cases lead to hyponatremia and "water intoxication (17) The blood brain barrier may also be damaged by toxins from infectious processes inside or outside the skull (22 50) Various drugs may act in the same way (4 53 68 76)

Suppression of adrenal cortical activity has been considered by many authors to be a pathogenetic mechanism of pseudotumor Thus Jefferson (42) and Dees & McKay Jr (14) regarded it as a probable mechanism in Addison s disease and Walker & Adamkiewicz (75) in long term steroid therapy states on the basis of their reviewed 24 cases Evidence of adrenal hypofunction was revealed by low 17-ketosteroids and 17 hydroxy ketosteroids in urine (14 75)

Later Oldstone (57) in six female patients with pseudotumor found normal levels of female sex hormones 17 ketosteroids and 17 hydroxy-corticosteroids in urine but no response to metopirone He then concluded that ovarian dysfunction per se plays no role in the pathogenesis which is instead a hypofunction of the pituitary ACTH adrenal axis due to failure in the synthesis or release of endogenous ACTH Whatever the mechanism the result is

a brain edema (60 75) which has been demonstrated at autopsy in cases of Addison s disease (42)

Dural sinus thrombosis is not considered to be guilty of interference with CSF absorption but to promote cerebral swelling (49 63 64 72 74) The causal relationship of superior longitudinal sinus thrombosis and pseudotumor—which is a common combination—can be most easily explained (74) namely by obstruction of bilateral venous outflow in the confluens sinuum There must be additional factors causing a bilateral obstruction when lateral sinus thrombosis is instead present These factors probably imply an interference with the by pass of CSF (79)

CLINICAL FEATURES IN INFANCY AND CHILDHOOD

Pseudotumor cerebri is an unusual condition in childhood Davidoff (12) reported 12 patients below 12 years of age among 81 cases and in a recent report from Guidetti *et al* (38) there were only 7 children 0–10 years of age among their 100 cases Purely pediatric series have been published among other authors by Eckler *et al* (17) and Rose & Matson (66)

The symptoms and signs (Table 5) are very similar from case to case and usually only differ in degree of severity There is however reason to differentiate between the clinical picture in infants and that in older children

The clinical picture in infancy is much more meager in symptoms and signs and more acute in its onset than in later years Characteristically a simple upper respiratory infection with or without antibiotic therapy has preceded the onset by a few days or a week A tense and bulging *fontanel may be the only sign but not* seldom in our experience the infant is also *irritable listless and dull This is in contrast to* the opinion of Bradshaw (6) who was impressed by the surprising alertness of the patients in association with their increased intracranial pressure Vomiting and feeding difficulties are common The head is usually not noticeably enlarged but widened sutures are regularly found within some days or weeks A cracked pot sound may be heard on percussion of the skull *Papilledema may or may not* be present

Table 5 *Clinical symptoms and signs in pseudotumor cerebri*

1 General symptoms
 Headache
 Nausea and vomiting
 Dizziness
 Tinnitus

2 Signs localized to the skull
 Bulging fontanel } in infants
 Suture diastasis } in infants
 Cracked pot sound } in infants

3 Ophthalmological signs
 Papilledema dilated veins
 Protruding discs
 Blurring of vision
 Visual hallucinations
 Diplopia
 Strabismus
 Visual field defects
 Nystagmus

4 Balance abnormalities
 Gait disturbance
 Other ataxic signs

5 Mental signs
 Apathy lethargy (seldom)
 Irritability
 Nervousness
 Disturbed memory

6 Convulsive signs
 General convulsions
 Focal convulsions (rare)

7 Other neurological signs
 VIth nerve palsy
 VIIth nerve weakness
 Facial numbness
 Pyramidal signs
 Speech defects

Older children complain of intermittent headache of usually moderate or even mild intensity *The headache is often localized to the* frontal regions Characteristically in the idio*pathic cases there have often been episodes of* less intense but the same sort of aching even *months or years before the eventual and more* multifarious picture has developed Nausea vomiting dizziness and tinnitus may be added in various degrees Visual impairment often appears early *distinguishing the condition* from genuine cerebral tumor When the first

symptoms of visual impairment appear a diagnosis of bilateral papilledema has nearly been made In severe cases it is connected with dilated veins of the ocular fundi hemorrhages and papillary protrusion up to 9 D (30) Weakened visual power may be the cause of nystagmus Diplopia and squint due to unilateral or more commonly bilateral VIth nerve palsy in addition to visual field defects make the patient feel unstable Gait disturbances result secondarily and these may be further increased by cerebellar ataxia and pyramidal disorders due to the brain lesion Focal neurological signs are only met exceptionally In only a few cases have facial weakness or numbness or speech defects been noted

Irritability nervousness or apathy may be observed also in older children Disorientation and disturbed memory is found in severe cases General convulsions may occur in 10–20 of older patients and more frequently in children (17) Focal fits are rare Acute states of increased blood pressure have also been described

Even in the presence of severe neurological symptoms and signs the general condition and the intellectual capacity in many patients remain unimpaired

A summary of complaints and clinical findings in our own material is shown in Table 1 Every patient with closed cranial sutures had protruded discs or at least unsharp disc edges and dilated veins in the ocular fundi In the other cases all infants bulging fontanels and suture diastasis were regularly found

SPECIAL CLINICAL EXAMINATIONS

Electroencephalographic examination shows normal findings in a high percentage of the cases (30 70) or reveals generalized changes with slow suppressed background activity and bilateral paroxysmal bursts (66) but no specific changes and no focal abnormalities (3)

Findings of normal constituents of the CSF are the rule A protein level of higher than 50 mg was only seen in 2 of 42 cases reported by Bradshaw (6) The CSF pressure is generally raised but is often considered to be lower than might be expected from the degree of disc protrusion in the ocular fundi Thus Guidetti *et al* (38) measured the pressure at lumbar level in 76 cases and found it normal in 39 moderately raised in 21 and more so only in 16 Surprising and marked fluctuations in CSF pressure from day to day were recorded by Bradshaw (6)

Air encephalography should according to definition give normal or near normal findings This was also the case in 69 of 100 patients in the recent series of Guidetti *et al* (38) These authors found small ventricles in 20 cases and slight symmetrical enlargements in 11 The same authors also strongly plead for the use of carotid angiography for diagnostic support They refer to a special angiographic pattern including a characteristic stretching and widening of the curve of the anterior cerebral artery in lateral views in the simultaneous absence of pneumographic evidence of expanding hydrocephalus This is in contrast to the findings of Goebell (30) who performed 42 carotid angiographies of a serial type illustrating all stages of cerebral circulation and assessed their 42 cases as normal

DIAGNOSIS

The most important point for an early diagnosis is awareness of the existence of this peculiar syndrome The diagnosis is then based on exclusion of localized expansive processes and on the history clinical findings and—if present—demonstration of hypertension of the CSF at lumbar or ventricular puncture Other supporting signs may be the demonstration of dural sinus thrombosis in cerebral angiography and signs of raised intracranial pressure in the plain skull X ray A normal or small ventricular size and a non-displaced ventricular system is essential EEG usually gives no support for the diagnosis

DIFFERENTIAL DIAGNOSIS

Pseudotumor cerebri is mainly a diagnosis of exclusion Therefore the importance lies on exhaustive studies to exclude all possible expansive or acute infectious intracranial processes in the cerebrum and the meninges (Table 6) *Hemispherical tumors* are usually diagnosed without difficulties cortical ones give rise to focal symptoms and signs at clinical examination focal changes in EEG and positive findings at carotid angiography Subcortical medial hemisphere tumors are best discovered by air encephalography Usually they deform and displace the ventricular system in addition to giving localizing signs, but they may provide difficulties when small and slowly growing Aneurysms are demonstrated by angiography, as well as hemorrhage which in addition commonly leads to loss of consciousness and bloody CSF

In infancy *subdural effusions* may give very similar clinical symptoms and signs Subdural taps and carotid angiography give a differentiation Echo encephalography may be of value in certain cases of subdural processes with abnormal lateral echoes When papilledema is present subdural effusions are characteristically often combined with peripapillary hemorrhages

Expansive hydrocephalus is readily diagnosed by demonstrating signs of raised intracranial pressure associated with markedly dilated ventricles

Encephalitis meningitis and *lead poisoning* usually give increased cells and/or proteins in CSF The clinical findings otherwise cannot be sharply delimited from those in benign intracranial hypertension *Vascular hypertension* can readily be excluded but one must remember that vascular hypertension may on the other hand occur secondarily to increased intracranial pressure also in cases of pseudotumor Bradycardia is then mostly associated

Particularly in generalized *craniosynostosis* the brain is secondarily compressed giving rise to strikingly bulging fontanels and/or papilledema, but the premature closure of sutures

Table 6 *Conditions of importance for differential diagnosis of pseudotumor cerebri*

1 Cerebral tumor
   Neoplasms
   Cysts aneurysms
   Hemorrhages
   Abscesses
2 Subdural processes
   Subdural hemorrhage
   Subdural effusions
3 *Expansive hydrocephalus*
   Obstructive forms
   Overproductive forms
4 Encephalitis
5 Meningitis
6 Lead poisoning
7 Vascular hypertension
8 Optic neuritis (or atrophy??)
9 Pseudo-papilledema in
   Hyperopia
   Thrombosis of central vein
   Deformed optic discs
10 Craniosynostosis
11 Constitutional pseudopapillitis

is readily diagnosed both clinically and with plain skull X rays

Isolated optic disc changes, especially *optic neuritis* may be of infectious or degenerative origin and may sometimes be diagnosed erroneously as pseudotumor with papilledema (11) Pathologic changes in CSF the early and severe impairment of vision and a failure to show a raised CSF pressure will together contribute towards a correct diagnosis Also other anomalies of the optic discs or eyeballs may mistakenly give rise to a suspicion of papilledema (28 31) but such conditions are usually isolated occasional findings and remain unchanged in follow up studies

TREATMENT

Pseudotumor cerebri is usually a benign condition which needs no therapy However careful observation is always needed especially in fulminant stages Diuretics, e g acetazolamide (37) or furcemide (66) or restriction of fluid and salt only seldom have any effect Pseudotumor induced by withdrawal of steroid or

ACTH is treated by a short term increase of the doses of steroids. Endocrine correcting therapy is of course the treatment of choice in hormonal hypo- or hyperfunctional conditions as well as adequate substitution in anemias and metabolic deficiency states.

Operative measures are mainly indicated when visual function is threatened. According to Holmes (39) loss of vision may be particularly suspected in the following situations (if primary eye disease has been excluded): 1) rapidly developing papilledema, 2) high grade papilledema (over 5 D) with retinal hemorrhages and exudates, 3) marked retinal arteriolar constriction, 4) narrowing of the visual fields and 5) brief periodic black-outs, even if papilledema is not severe. Repeated lumbar punctures can only temporarily correct such conditions (6). Therefore subtemporal decompression or nowadays preferably shunt operations are highly recommended in impending visual loss. Excision of occluding sinus thrombi can be performed in selected cases.

COURSE AND DURATION OF ILLNESS

The duration of the pseudotumorous state may vary from some days up to some few years. However, most of the cases are free from papilledema 6 months after the onset (66). Other symptoms and signs of pseudotumor have a still shorter duration. Recurrence are uncommon particularly in childhood. Permanent impairment of vision may remain in severe cases but the outcome in general is considered in the literature to be good or even excellent (13, 37). The most recent follow up study was made by Guidetti *et al* (38). All of his 80 patients had become free from neurological symptoms and signs. Twelve cases had a residual visual defect. We had the same good experiences from our series. No deaths were recorded. A definitely impaired vision was only noted in the most severe of our cases (case 3) and three others had some slight residual neurological abnormalities. All the rest had completely recovered.

SUMMARY

Clinical experiences from 18 infants and children with a diagnosis of pseudotumor cerebri and cared for in Uppsala and Turku during the years 1960–69 have been summarized. Three case reports with clinical pictures of particular interest have been given in more detail. In addition, a general review of this topic has been given.

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REFERENCES

1 Barr, D. P., McBryde, C. M. & Sanders, T. E.: Tetany with increased intracranial pressure and papilledema. *Trans Ass Amer Physicians* 53: 227, 1938.

2 Bass, M. H. & Caplan, J.: Vitamin A deficiency in infancy. *J Pediat* 47: 690, 1955.

3 Beller, A. J.: Benign post-traumatic intracranial hypertension. *J Neurol Neurosurg Psychiat* 27: 149, 1964.

4 Boreus, L. O. & Sundstrom, B.: Intracranial hypertension in a child during treatment with nalidixic acid. *Brit Med J* II: 744, 1967.

5 Boudin, G., Funck-Brentano, J. L. & Guyno, M.: Considerations sur une encephalopathie addisonienne. *Bull Soc Méd Hôp Paris* 66: 1741, 1950.

6 Bradshaw, P.: Benign intracranial hypertension. *J Neurol Neurosurg Psychiat* 19: 28, 1956.

7 Buchheit, W. A., Burton, C., Haag, B. & Shaw, D.: Papilledema and idiopathic intracranial hypertension, report of a familial occurrence. *New Eng J Med* 280: 938, 1969.

8 Calcagno, P. L. & Rubin, M. I.: Physiologic considerations concerning corticosteroid therapy and complications in the nephrotic syndrome. *J Pediat* 58: 686, 1961.

9 Chaptal, J., Jean, R. & Bonnet, H.: Syndrome pseudotumoral mortel par deprivation surrenale. Tableau d'hypertension cérébrale fatale au cours d'une nephrose lipoidique avec arret brusque du traitment cortisonique. *Acta Neuropath* 10: 45, 1968.

10 Cornfeld, D. & Cooke, R. E.: Vitamin A deficiency: case report. Unusual manifestations in a 5 1/2 month old baby. *Pediatrics* 10: 33, 1952.

11 Dandy, W. E.: Intracranial pressure without brain tumor. *Ann Surg* 106: 492, 1937.

12 Davidoff, L. M.: Pseudotumor cerebri. Benign intracranial hypertension. *Neurology* 6: 605, 1956.

13 Davidoff L M & Dyke C G A presentation of a series of cases of serous meningitis *J Nerv Ment Dis 83* 700 1936
14 Dees S C & McKay H W Jr Occurrence of pseudotumor cerebri (benign intracranial hypertension) during treatment of children with asthma by adrenal steroids Report of three cases *Pediatrics 23 1143 1959*
15 Devenney J W Jr & Shea J H Allergic encephalopathy with papilledema *Arch Neurol Psychiat 68* 791 1952
16 DiGeorge A M Hypoparathyroidism *In* W E Nelson (ed) *Textbook of Pediatrics* W B Saunders Company Philadelphia 1964 p 1284
17 Eckler E Schonberg D & Bierich J R Pseudotumor cerebri infolge Corticosteroidtherapie *Mschr Kinderheilk 114* 271 1966
18 *Eiben R M Acute brain swelling (toxic encephalopathy) Pediat Clin N America 14* 797 1967
19 Erwner M S & Myers D Otitic hydrocephalus with a suggestion as to etiology *Ann Otol 45* 553 1936
20 Fields J P Bulging fontanel a complication of tetracycline therapy in infants *J Pediat 58* 74 1961
21 Fleury D D P Verhoogde intracraniele druk als eerste symptoom bij een kind met acute leukemie *Nederl T Geneesk 112* 1170 1968
22 Foley J Benign forms of intracranial hypertension— toxic and otitic hydrocephalus *Brain 78* 1 1955
23 Ford F R & Murphy E L Increased intracranial pressure A clinical analysis of the causes and characteristics of several types *Bull Hopkins Hosp 64* 369 1939
24 Ford F R & Walsh F B Guillain Barre syndrome (acute infective polyneuritis) with increased intracranial pressure and papilledema *Bull Hopkins Hosp 73 391 1943*
25 Fraser D Yendt E R & Christie F H E Metabolic abnormalities in hypophosphatasia *Lancet 1* 286 1955
26 Foldi M Obál F Kahan A Wagner A Csanda E & Borcsok E Lymphogene Encephalopathie *Acta Paediat Acad Sci Hung 8* 171 1967
27 Gaisford W Some diagnostic difficulties *J Pediat 56* 259 1960
28 Gass H H Papilledema and pseudotumor cerebri following poliomyelitis *Amer J Dis Child 93* 640 1957
29 Glowacki J Guazzi G C Alvisi C Gambetti P Jonckheer M & Tassinari C A L'oedème cérébral pseudotumoral endocrinien et ou metabolique *Acta Neurol Belg 65* 873 1965
30 Goebell H Die Stauungspapille unklarer Genese Ein Beitrag zum Bild des Pseudotumor cerebri *Fortschr Neurol Psychiat 32* 265 1964
31 Gordon R C & Kelsey W M Pseudotumor cerebri in congenital adrenal hyperplasia *Amer J Dis Child 113* 727 1967
32 Granstrom K O Stasapapill papillodem opticusneurit papillit pseudopapillit *Opusc M (Stockh) 10* 3 1965
33 Greer M Benign intracranial hypertension Following corticosteroid therapy *Neurology* 439 1963
34 — Benign intracranial hypertension IV Menarche *Neurology 14* 569 1964
35 — Benign intracranial hypertension V Menstrual dysfunction *Neurology 14* 668 1964
36 — Benign intracranial hypertension VI Obes *Neurology 15* 382 1965
37 — Benign intracranial hypertension (pseudotumor cerebri *Pediat Clin N America 14* 1967
38 Guidetti B Giuffre R & Gambacorta, Follow up study of 100 cases of pseudotumor cerebri *Acta Neurochir 18* 259 1968
39 Holmes G The prognosis in papilledema *J Ophthal 21* 337 1937
40 Ikkala E & Laitinen L Papilloedema due iron deficiency anaemia *Acta Haemat 29* 3 1963
41 Jacobson H G & Shapiro J H Pseudotum cerebri *Radiology 82* 202 1964
42 Jefferson A A clinical correlation between encephalopathy and papilloedema in Addison's disease *J Neurol Neurosurg Psychiat 19* 21 195
43 Jelke H Hypophosphatasia *Acta Paediat Scan 49* 297 1960
44 Kehrer F A Zur Frage des Pseudotumor cerebri *Deutsch Z Nervenheilk 160* 1 1949
45 Lecks H I & Baker D Pseudotumor cereb an allergic phenomenon? A discussion of 17 cas including two of infants manifesting pseudotumor while receiving soybean feedings *Clin Pedi (Phila) 4* 32 1965
46 Loman J & Dameshek W Increased intracranial venous and cerebrospinal fluid pressures polycythemia *Trans Amer Neurol Ass 70* 1944
47 Lubeck M J Papilledema caused by iron-deficiency anaemia *Trans Amer Acad Ophthal Oto laryng 63* 306 1959
48 *Marie J & See G* Acute hypervitaminosis A of the infant Its clinical manifestation with benign acute hydrocephalus and pronounced bulge of the fontanel a clinical and biologic study *Amer Dis Child 87* 731 1954
49 Martin J P & Sheehan H L Primary thrombosis of cerebral veins (following childbirth) *Brit Med J 1* 349 1941
50 McAlpine D Toxic hydrocephalus *Brain 60* 180 1937
51 McCullagh E P Menstrual edema with intracranial hypertension (pseudotumor cerebri) Report of a case *Cleveland Clin Quart 8* 70 1941
52 Millichap J G Benign intracranial hypertension and otitic hydrocephalus *Pediatrics 23* 257 1959
53 Murahovschi J Scaff A de Andrade A J R Goldman L Racowski P & Crespin J Trata

mento das diarréias agudas da primeira infancia com o ácido nalidíxico *Pediat Prát (S Paulo) 37* 31 1966

54 Murray R G & Walsh F B Ocular abnormalities in poliomyelitis and their pathogenesis *Canad Med Ass J 70* 141 1954

55 Nonne M Der Pseudotumor cerebri *Neue Dtsch Clin 10* 107 1914

56 O Doherty N J Acute benign intracranial hypertension in an infant receiving tetracycline *Develop Med Child Neurol 7* 677 1965

57 O'disteme M B A The endocrinological aspects of benign intracranial hypertension *Arch Neurol 15* 362 1966

58 Oski F A Roseola infantum another cause of bulging fontanel *Amer J Dis Child 101* 376 1961

59 Palmer R F Searles H H & Boldrey E B Papilledema and hypoparathyroidism simulating brain tumor *J Neurosurg 16* 378 1959

60 Paterson R DePasquale N & Mann S Pseudotumor cerebri *Medicine 40* 85 1961

61 Persson B Tunell R & Ekengren K Chronic vitamin A intoxication during the first half year of life Description of 5 cases *Acta Paediat Scand 54* 9 1965

62 Quincke H Ueber meningitis serosa *Samml Klin Vortr Inn Med 23* 655 1893

63 Ray B S & Dunbar H S Thrombosis of the superior sagittal sinus as a cause of pseudotumor cerebri methods of diagnosis and treatment *Trans Amer Neurol Ass 75* 12 1950

64 — Thrombosis of the dural venous sinuses as a cause of pseudotumor cerebri *Ann Surg 134* 376 1951

65 Reid H A & Harris W Reversible papilloedema in pernicious anaemia *Brit Med J 1* 20 1951

66 Rose A & Matson D D Benign intracranial hypertension in children *Pediatrics 39* 227 1967

67 Sahs A L & Joynt R J Brain swelling of unknown cause *Neurology 6* 791 1956

68 Schmitt B D & Krivit W Benign intracranial hypertension associated with a delayed penicillin reaction *Pediatrics 43* 50 1969

69 Schwaber J R & Blumberg A G Papilledema associated with blood loss anaemia *Ann Intern Med 55* 1004 1961

70 Silverman D Papilledema and the electroencephalogram *Neurology 14* 899 1964

71 Sutphin A Albright F & McCune D J Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moniliasis *J Clin Endocr 3* 625 1943

72 Symonds C P Otitic hydrocephalus *Brain 54* 55 1931

73 Valentine G H Triamcinolone and intracranial hypertension side effect? *Lancet 1* 892 1959

74 Wagener H P Pseudotumor cerebri *Amer J Med Sci 227* 214 1954

75 Walker A E & Adamkiewicz J J Pseudotumor cerebri associated with prolonged corticosteroid therapy Reports of four cases *JAMA 188* 779 1964

76 Walker S H Balamo I & Standiford W E Nalidixic acid in childhood urinary infection clinical and laboratory experience with fifty patients *Clin Pediat 5* 718 1966

77 Walsh F B Clark D B Thompson R S & Nicholson D H Oral contraceptives and neuro-ophthalmologic interest *Arch Ophthal 74* 628 1965

78 Wilson C B & Bertan V A review of factors in the pathogenesis of pseudotumor cerebri *Turk J Pediat 9* 125 1967

79 Woodhall B Variations of cranial sinuses in region of torcular herophili *Arch Surg 33* 297 1936

80 Yaskin J C Groff R A & Shenkin H A Severe papilledema in diffuse infections of the nervous system *Trans Amer Neurol Ass 72* 21 1947

81 Zuidema G D & Cohen S J Pseudotumor cerebri *J Neurosurg 11* 433 1954

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14 Dees S C & McKay H W Jr Occurrence of pseudotumor cerebri (benign intracranial hypertension) during treatment of children with asthma by adrenal steroids Report of three cases *Pediatrics 23* 1143 1959
15 Devenney J W Jr & Shea J H Allergic encephalopathy with papilledema *Arch Neurol Psychiat 68* 791 1952
16 DiGeorge A M Hypoparathyroidism *In* W E Nelson (ed) *Textbook of Pediatrics* W B Saunders Company Philadelphia 1964 p 1284
17 Eckler E Schonberg D & Brench J R Pseudotumor cerebri infolge Corticosteroidtherapie *Mschr Kinderheilk 114* 271 1966
18 Eiben R M Acute brain swelling (toxic encephalopathy) *Pediat Clin N America 14* 797 1967
19 Ersner M S & Myers D Otitic hydrocephalus with a suggestion as to etiology *Ann Otol 45* 553 1936
20 Fields J P Bulging fontanel a complication of tetracycline therapy in infants *J Pediat 59* 74 1961
21 Fleury D D P Verhoogde intracranieele druk als eerste symptoom bij een kind met acute leukemie *Nederl T Geneesk 112* 1170 1968
22 Foley J Benign forms of intracranial hypertension— toxic and otitic" hydrocephalus *Brain 78* 1 1955
23 Ford F R & Murphy E L Increased intracranial pressure A clinical analysis of the causes and characteristics of several types *Bull Hopkins Hosp 64* 369 1939
24 Ford F R & Walsh F B Guillain Barré syndrome (acute infective polyneuritis) with increased intracranial pressure and papilledema *Bull Hopkins Hosp 73* 391 1943
25 Fraser D Yendt E R & Christie F H E Metabolic abnormalities in hypophosphatasia *Lancet 1* 286 1955
26 Foldi M Obál F Kahán A Wagner A Csanda E & Borcsok E Lymphogene Enzephalopathie *Acta Paediat Acad Sci Hung 8* 171 1967
27 Gainford W Some diagnostic difficulties *J Pediat 56* 259 1960
28 Gass H H Papilledema and pseudotumor cerebri following poliomyelitis *Amer J Dis Child 93* 640 1957
29 Glowacki J Guazzi G C Alvisi C Gambetti P Jonckheer M & Tassinari C A L'oedème cérébral pseudotumoral endocrinien et ou métabolique *Acta Neurol Belg 65* 873 1965
30 Goebell H Die Stauungspapille unklarer Genese Ein Beitrag zum Bild des Pseudotumor cerebri *Fortschr Neurol Psychiat 32* 265 1964
31 Gordon R C & Kelsey W M Pseudotumor cerebri in congenital adrenal hyperplasia *Amer J Dis Child 113* 727 1967
32 Granstrom K O Stasspapill papillodem opticusoedem papillit pseudopapillit *Opusc Med* (Stockh) *10* 3 1965
33 Greer M Benign intracranial hypertension II Following corticosteroid therapy *Neurology 13* 439 1963
34 — Benign intracranial hypertension IV Menarche *Neurology 14* 569 1964
35 — Benign intracranial hypertension V Menstrual dysfunction *Neurology 14* 668 1964
36 — Benign intracranial hypertension VI Obesity *Neurology 15* 382 1965
37 — Benign intracranial hypertension (pseudotumor cerebri *Pediat Clin N America 14* 819 1967
38 Guidetti B Giuffre R & Gambacorta D Follow up study of 100 cases of pseudotumor cerebri *Acta Neurochir 18* 259 1968
39 Holmes G The prognosis in papilledema *Brit J Ophthal 21* 337 1937
40 Ikkala E & Laitinen L Papilloedema due to iron deficiency anaemia *Acta Haemat 29* 368 1963
41 Jacobson H G & Shapiro J H Pseudotumor cerebri *Radiology 82* 202 1964
42 Jefferson A A clinical correlation between encephalopathy and papilloedema in Addison's disease *J Neurol Neurosurg Psychiat 19* 21 1956
43 Jelke H Hypophosphatasia *Acta Paediat Scand 49* 297 1960
44 Kehrer F A Zur Frage des Pseudotumor cerebri" *Deutsch Z Nervenheilk 160* 1 1949
45 Lecks H I & Baker D Pseudotumor cerebri an allergic phenomenon? A discussion of 17 cases including two of infants manifesting pseudotumor while receiving soybean feedings *Clin Pediat* (Phila) *4* 32 1965
46 Loman J & Damashek W Increased intracranial venous and cerebrospinal fluid pressures in polycythemia *Trans Amer Neurol Ass 70* 84 1944
47 Lubeck M J Papilledema caused by iron-deficiency anemia *Trans Amer Acad Ophthal Otolaryng 63* 306 1959
48 Marie J & See G Acute hypervitaminosis A of the infant Its clinical manifestation with benign acute hydrocephalus and pronounced bulge of the fontanel a clinical and biologic study *Amer J Dis Child 87* 731 1954
49 Martin J P & Sheehan H L Primary thrombosis of cerebral veins (following childbirth) *Brit Med J 1* 349 1941
50 McAlpine D Toxic hydrocephalus *Brain 60* 180 1937
51 McCullagh E P Menstrual edema with intracranial hypertension (pseudotumor cerebri) Report of a case *Cleveland Clin Quart 8* 202 1941
52 Millichap J G Benign intracranial hypertension and otitic hydrocephalus *Pediatrics 23* 257 1959
53 Murahovschi J Scaff A de Andrade A J R Goldman L Racowski P & Creston J Trata

mento das diarreias agudas da primeira infancia com o acido nalidixico *Pediat Prat* (S Paulo) 37 31 1966

54 Murray R G & Walsh F B Ocular abnormalities in poliomyelitis and their pathogenesis *Canad Med Ass J* 70 141 1954

55 Nonne M Der Pseudotumor cerebri *Neue Dtsch Chir* 10 107 1914

56 O Doherty N J Acute benign intracranial hypertension in an infant receiving tetracycline *Develop Med Child Neurol* 7 677 1965

57 Oldstone M B A The endocrinological aspects of benign intracranial hypertension *Arch Neurol* 15 362 1966

58 Oski F A Roseola infantum another cause of bulging fontanel *Amer J Dis Child* 101 376 1961

59 Palmer R F Searles H H & Boldrey E B Papilledema and hypoparathyroidism simulating brain tumor *J Neurosurg* 16 378 1959

60 Paterson R DePasquale N & Mann S Pseudotumor cerebri *Medicine* 40 85 1961

61 Persson B Tunell R & Ekengren K Chronic vitamin A intoxication during the first half year of life Description of 5 cases *Acta Paediat Scand* 54 49 1965

62 Quincke H Ueber meningitis serosa *Samml Klin Vortr Inn Med* 23 655 1893

63 Ray B S & Dunbar H S Thrombosis of the superior sagittal sinus as a cause of pseudotumor cerebri methods of diagnosis and treatment *Trans Amer Neurol Ass* 75 12 1950

64 — Thrombosis of the dural venous sinuses as a cause of pseudotumor cerebri *Ann Surg* 134 376 1951

65 Reid H A & Harris W Reversible papilloedema in percutaneous infusion *Brit Med J* 1 20 1951

66 Rose A & Matson D D Benign intracranial hypertension in children *Pediatrics* 39 227 1967

67 Sahs A L & Joynt R J Brain swelling of unknown cause *Neurology* 6 791 1956

68 Schenitt B D & Krivit W Benign intracranial hypertension associated with a delayed penicillin reaction *Pediatrics* 43 50 1969

69 Schwaber J R & Blumberg A G Papilloedema associated with blood loss anaemia *Ann Intern Med* 55 1004 1961

70 Silverman D Papilledema and the electroencephalogram *Neurology* 14 899 1964

71 Sutphin A Albright F & McCune D J Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moniliasis *J Clin Endocr* 3 625 1943

72 Symonds C P Otitic hydrocephalus *Brain* 54 55 1931

73 Valentine G H Triamcinolone and intracranial hypertension side effect? *Lancet* 1 892 1959

74 Wagener H P Pseudotumor cerebri *Amer J Med Sci* 227 214 1954

75 Walker A E & Adamkiewicz, J J Pseudotumor cerebri associated with prolonged corticosteroid therapy Reports of four cases *JAMA* 188 779 1964

76 Walker S H, Salamo I & Standiford W F Nalidixic acid in childhood urinary infection clinical and laboratory experience with fifty patients *Clin Pediat* 5 718 1966

77 Walsh F B Clark D B Thompson R S & Nicholson D H Oral contraceptives and neuro-ophthalmologic interest *Arch Ophthal* 74 628 1965

78 Wilson C B & Bertan V A review of factors in the pathogenesis of pseudotumor cerebri *Turk J Pediat* 9 125 1967

79 Woodhall B Variations of cranial sinuses in region of torcular herophili *Arch Surg* 33 297 1936

80 Yaskin J C Groff R A & Shenkin H A Severe papilledema in diffuse infections of the nervous system *Trans Amer Neurol Ass* 72 21 1947

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16 DiGeorge A M Hypoparathyroidism *In* W E Nelson (ed) *Textbook of Pediatrics* W B Saunders Company Philadelphia 1964 p 1284
17 Eckler E Schonberg D & Bierich J R Pseudotumor cerebri infolge Corticosteroidtherapie *Mschr Kinderheilk 114* 271 1966
18 Eiben R M Acute brain swelling (toxic encephalopathy) *Pediat Clin N America 14* 797 1967
19 Erzner M S & Myers D Otitic hydrocephalus with a suggestion as to etiology *Ann Otol 45* 553 1936
20 Fields J P Bulging fontanel a complication of tetracycline therapy in infants *J Pediat 58 74* 1961
21 Fleury D D P Verhoogde intracraniele druk als eerste symptoom bij een kind met acute leukemie *Nederl T Geneesk 112* 1170 1968
22 Foley J Benign forms of intracranial hypertension— toxic and otitic hydrocephalus *Brain 78* 1 1955
23 Ford F R & Murphy E L Increased intracranial pressure A clinical analysis of the causes and characteristics of several types *Bull Hopkins Hosp 64* 369 1939
24 Ford F R & Walsh F B Guillain Barre syndrome (acute infective polyneuritis) with increased intracranial pressure and papilledema *Bull Hopkins Hosp 73* 391 1943
25 Fraser D Yendt E R & Christie F H E Metabolic abnormalities in hypophosphatasia *Lancet 1* 286 1955
26 Foldi M Obál F Kahan A Wagner A Csanda E & Boresok E Lymphogene Enzephalopathie *Acta Paediat Acad Sci Hung 8* 171 1967
27 Gaisford W Some diagnostic difficulties *J Pediat 56* 259 1960
28 Gass H H Papilledema and pseudotumor cerebri following poliomyelitis *Amer J Dis Child 93* 640 1957
29 Glowicki J Guzzi G C Alvisi C Gambetti P Jonckheer M & Tasinari C A L oedeme cerebral pseudotumoral endocrinien et ou metabolique *Acta Neurol Belg 65* 873 1965
30 Goebell H Die Stauungspapille unklarer Genese Ein Beitrag zum Bild des Pseudotumor cerebri *Fortschr Neurol Psychiat 32* 265 1964
31 Gordon R C & Kelsey W M Pseudotumor cerebri in congenital adrenal hyperplasia *Amer J Dis Child 113* 727 1967
32 Granstrom K O Stasapapill papillodem opticusneurit papillit pseudopapillit *Opusc Med* (Stockh) *10* 3 1965
33 Greer M Benign intracranial hypertension II Following corticosteroid therapy *Neurology 13* 439 1963
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37 — Benign intracranial hypertension (pseudotumor cerebri *Pediat Clin N America 14* 819 1967
38 Guidetti B Giuffre R & Gambacorta D Follow up study of 100 cases of pseudotumor cerebri *Acta Neurochir 18* 259 1968
39 Holmes G The prognosis in papilledema *Brit J Ophthal 21* 337 1937
40 Ikkala E & Laitinen L Papilloedema due to iron deficiency anaemia *Acta Haemat 29* 368 1963
41 Jacobsson H G & Shapiro J H Pseudotumor cerebri *Radiology 82* 207 1964
42 Jefferson A A clinical correlation between encephalopathy and papilloedema in Addison s disease *J Neurol Neurosurg Psychiat 19 21 1956*
43 Jelke H Hypophosphatasia *Acta Paediat Scand 49* 297 1960
44 Kehrer F A Zur Frage des Pseudotumor cerebri *Deutsch Z Nervenheilk 160* 1 1949
45 Lecks H I & Baker D Pseudotumor cerebri an allergic phenomenon? A discussion of 17 cases including two of infants manifesting pseudotumor while receiving soybean feedings *Clin Pediat* (Phila) *4* 32 1965
46 Loman J & Dameshek W Increased intracranial venous and cerebrospinal fluid pressures in polycythemia *Trans Amer Neurol Ass 70 84* 1944
47 Lubeck M J Papilledema caused by iron-deficiency anaemia *Trans Amer Acad Ophthal Otolaryng 63* 306 1959
48 Marie J & See G Acute hypervitaminosis A of the infant Its clinical manifestation with benign acute hydrocephalus and pronounced bulge of the fontanel a clinical and biologic study *Amer J Dis Child 87* 731 1954
49 Martin J P & Sheehan H L Primary thrombosis of cerebral veins (following childbirth) *Brit Med J 1* 349 1941
50 McAlpine D Toxic hydrocephalus *Brain 60* 180 1937
51 McCullagh E P Menstrual edema with intracranial hypertension (pseudotumor cerebri) Report of a case *Cleveland Clin Quart 8* 202 1941
52 Millichap J G Benign intracranial hypertension and otitic hydrocephalus *Pediatrics 23* 257 1959
53 Murahovschi J Scaff A de Andrade A Goldman L Racowski P & Crespin J

of the chest showed no abnormality A nasal swab was taken and she was started on a course of tetracycline Two days after the onset of the upper respiratory infection she suddenly collapsed and died

Necropsy confirmed the diagnosis of osteogenesis imperfecta and death was considered to be due to the presence of a large subdural haematoma

Case 2

R O male one of twins was born in U C H on 31 1 67 at 36 weeks to a young primigravida He is the second twin and weighed 1800 g at birth The skull showed asymmetry with multiple defects and large anterior fontanelle The lower legs were short and bowed and there was evidence of fractures of both tibiae and fibulae The sclerae were blue X ray of skull showed multiple wormian bones and radiological evidence of hydrocephalus X ray of the legs showed marked bowing as previously noted in the film taken during pregnancy and fracture of right femur He made satisfactory progress and on discharge on 26 2 68 was weighing 2210 g At follow up there was no major difficulty although it was noted that weight gain had been much slower than the unaffected twin At the age of 18 months he has not stood nor walked Haemoglobin genotype is AS

The twin brother weighed 3100 g at birth with a head circumference of 35 6 cm Examination of all the systems was completely normal He became moderately icteric in the first week of life but he required no special treatment On discharge he weighed 4000 g This twin died suddenly from unknown cause but suspected to be a complication of sickle cell disease at a time when full investigation of the twins for zygosity was being arranged

Family history Mother is 160 cm tall Pregnancy was normal and the reason for hospital delivery was twin pregnancy and the suspected abnormality in one of the twins There was no physical defect detected on examination and the Haemoglobin genotype is AS

Father is a short man (height 130 35 cm) with a relatively large head and faint bluish sclerae with brownish pigmentation He gave no history of fractures at any time Haemoglobin genotype is also reported as AS

DISCUSSION

Osteogenesis imperfecta is infrequently seen in Nigeria In a five year period at the University College Hospital Ibadan two definite cases have been previously diagnosed One was a four year-old male child who presented with multiple fractures of tibia and fibula with gross deformity dwarfing and frontal bossing He had no bluish sclerae The second was a one week-old male baby who presented at birth with limb deformities and blue sclera Clinical and radiological examinations established osteogenesis imperfecta This baby died suddenly at the age of 30 days

The cases reported here are one of two pairs of like sexed twins but no pair of twins could be more dissimilar in appearance However complete haematological and other investigations to determine zygosity were not possible in these present case reports The limited available information would suggest that the parents (mother in 1st father in 2nd) are heterozygous carriers and the condition in the babies was transmitted as a recessive characteristic

The work of Smars (8) however showed that majority of reported cases of osteogenesis imperfecta were due to dominant autosomal gene but there are cases which do not fall into this type of inheritance Into the latter group fall cases where the disease have missed a generation or there are no demonstrable osteogenesis imperfecta in the parents or other sibs These latter cases can be explained on the basis of complete suppression in one generation or the mutation of the gene in the offsprings

There are few reports of osteogenesis imperfecta in twins Zeitoun *et al* (10) reported for the first time the condition in dizygotic twins both of whom were affected The parents were free of any trait of the disease Other reports have been in siblings Seedorff (7) reported 180 affected members of 55 Danish families and in six of these both parents were normal and found seven cases of the congenital type He concluded from this study that the condition was inherited as a result of a dominant mutant gene

Heys *et al* (5) emphasized the association of osteogenesis imperfecta and odontogenesis imperfecta which existed in 18 families studied There were only 2 cases of the congenital form in this series Freda *et al* (3) in a review of the literature found 90 cases to which they added 16 of their own Sarma (6) encountered one case among 543 abnormal babies Chawla (2) reported one family in which there were

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CASE REPORT

OSTEOGENESIS IMPERFECTA CONGENITA IN ONE OF TWINS

O SERIKI

From the Department of Paediatrics University College Hospital Ibadan Nigeria

Osteogenesis imperfecta is characterised by increased fragility of bones, spontaneous or slight traumatic fractures Associated features are blue sclerae and otosclerosis in adult life There are various types of the condition based on time of onset and severity

The congenital type was first described by Vrolik in 1849 (9) This type presents a characteristic clinical picture and represents the extreme example of the disease In this type fracture deformities may occur in utero or the bones may break during parturition Many victims of the disease are stillborn or succumb shortly after birth

The mode of inheritance of this condition has not been clearly defined Goldfarb & Ford (4) reported two consecutive female siblings with congenital osteogenesis imperfecta one of whom lived to the age of 5 months and the other beyond 5 months Both had blue sclerae and evidence of fractures in utero with further fractures occurring after birth Awwaad & Reda (1) described two similar cases of congenital osteogenesis imperfecta in one sibship More recently Zeitoun *et al* (10) described the same condition in dizygotic twins The two infants survived for 2 1/ months and 4 months respectively and both died from bronchopneumonia

Further examples of osteogenesis imperfecta congenita in one of two sets of twins are now reported This is probably the first report of this condition from West Africa

Case I

Twin I (U C H No 196998) a female was admitted to University College Hospital (U C H) at the age of 17 hours on account of her small size and limb deformities She was born at home on a farm after an apparent normal pregnancy and labour

Physical examination showed a small premature baby weighing 1374 g The head was globular and showed very marked softening of the cranial bones The anterior and posterior fontanelles were wide and slightly tense The eyes were bulging and the sclerae blue There were multiple fracture deformities of the four limbs giving the infant a characteristic appearance There was no other abnormality in all the systems

Radiographs showed radiological features of osteogenesis imperfecta with fractures of all limbs and ribs

Laboratory findings were Serum calcium 8 8 mg per 100 ml inorganic phosphate 5 9 mg per 100 ml alkaline phosphatase 19 K A units Serum urea 24 mg per 100 ml and normal serum electrolytes

Twin II weighed 1322 g on admission Clinical and radiological examinations were completely normal She thrived well and on discharge thirty days after had gained 653 g

Family history The mother was a young lady with the only abnormal finding of bluish sclerae The father was not available for examination but detailed enquiries from the wife did not suggest abnormality in the father or other members of the family A 3 year old sibling of the case was healthy and normal

Management The baby was nursed in an incubator and fed by a naso gastric tube She had a course of prophylactic penicillin and anti tetanus serum Progress was satisfactory until twenty one days after admission when she developed a cold but examination

of the chest showed no abnormality A nasal swab was taken and she was started on a course of tetracycline Two days after the onset of the upper respiratory infection she suddenly collapsed and died

Necropsy confirmed the diagnosis of osteogenesis imperfecta and death was considered to be due to the presence of a large subdural haematoma

Case 3

B O male one of twins was born in U C H on 31 12 67 at 36 weeks to a young primigravida He is the second twin and weighed 1800 g at birth The skull showed asymmetry with multiple defects and large anterior fontanelle The lower legs were short and bowed and there was evidence of fractures of both tibiae and fibulae The sclerae were blue X ray of skull showed multiple wormian bones and radiological evidence of hydrocephalus X ray of the legs showed marked bowing as previously noted in the film taken during pregnancy and fracture of right femur He made satisfactory progress and on discharge on 26 2 68 was weighing 2270 g At follow up there was no major difficulty although it was noted that weight gain had been much slower than the unaffected twin At the age of 18 months he has not stood nor walked Haemoglobin genotype is AS

The twin brother weighed 3100 g at birth with a head circumference of 35 6 cm Examination of all the systems was completely normal He became moderately icteric in the first week of life but he required no special treatment On discharge he weighed 4000 g This twin died suddenly from unknown cause but suspected to be a complication of sickle cell disease at a time when full investigation of the twins for zygosity was being arranged

Family history Mother is 160 cm tall Pregnancy was normal and the reason for hospital delivery was twin pregnancy and the suspected abnormality in one of the twins There was no physical defect detected on examination and the Haemoglobin genotype is AS

Father is a short man (height 130 93 cm) with a relatively large head and faint bluish sclerae with brownish pigmentation He gave no history of fractures at any time Haemoglobin genotype is also reported as AS

DISCUSSION

Osteogenesis imperfecta is infrequently seen in Nigeria In a five year period at the University College Hospital Ibadan two definite cases have been previously diagnosed One was a four year-old male child who presented with multiple fractures of tibia and fibula with gross deformity dwarfing and frontal bossing He had no bluish sclerae The second was a one week-old male baby who presented at birth with limb deformities and blue sclera Clinical and radiological examinations established osteogenesis imperfecta This baby died suddenly at the age of 30 days

The cases reported here are one of two pairs of like sexed twins but no pair of twins could be more dissimilar in appearance However complete haematological and other investigations to determine zygosity were not possible in these present case reports The limited available information would suggest that the parents (mother in 1st father in 2nd) are heterozygous carriers and the condition in the babies was *transmitted* as a recessive characteristic

The work of Smårs (8) however showed that majority of reported cases of osteogenesis imperfecta were due to dominant autosomal gene but there are cases which do not fall into this type of inheritance Into the latter group fall cases where the disease have missed a generation or there are no demonstrable osteogenesis imperfecta in the parents or other sibs These latter cases can be explained on the basis of *complete* suppression in one generation or the mutation of the gene in the offsprings

There are few reports of osteogenesis imperfecta *in twins* Zeitoun *et al* (10) reported for the first time the condition in dizygotic twins both of whom were affected The parents were free of any trait of the disease Other reports have been in siblings Seedorff (7) reported 180 affected members of 55 Danish families and in six of these both parents were normal and found seven cases of the congenital type He concluded from this study that the condition was inherited as a *result* of a dominant mutant gene

Heys *et al* (5) emphasised the association of osteogenesis imperfecta and odontogenesis imperfecta which existed in 18 families studied There were only 2 cases of the congenital form *in this series* Freda *et al* (3) in a review of the literature found 90 cases to which they added 16 of their own Sarma (6) encountered one case among 543 abnormal babies Chawla (2) reported one family in which there were

Acta Pædiat Scand 59 340–342, 1970

CASE REPORT

OSTEOGENESIS IMPERFECTA CONGENITA IN ONE OF TWINS

O SERIKI

From the Department of Paediatrics University College Hospital Ibadan Nigeria

Osteogenesis imperfecta is characterised by increased fragility of bones, spontaneous or slight traumatic fractures Associated features are blue sclerae and otosclerosis in adult life There are various types of the condition based on time of onset and severity

The congenital type was first described by Vrolik in 1849 (9) This type presents a characteristic clinical picture and represents the extreme example of the disease In this type fracture deformities may occur in utero or the bones may break during parturition Many victims of the disease are stillborn or succumb shortly after birth

The mode of inheritance of this condition has not been clearly defined Goldfarb & Ford (4) reported two consecutive female siblings with congenital osteogenesis imperfecta one of whom lived to the age of 5 months and the other beyond 5 months Both had blue sclerae and evidence of fractures in utero with further fractures occurring after birth Awwaad & Reda (1) described two similar cases of congenital osteogenesis imperfecta in one sibship More recently Zeitoun *et al* (10) described the same condition in dizygotic twins The two infants survived for 2 ¹/ months and 4 months respectively and both died from bronchopneumonia

Further examples of osteogenesis imperfecta congenita in one of two sets of twins are now reported This is probably the first report of this condition from West Africa

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four affected sıblings—3 males and 1 female Remaınıng 3 sıblings as well as the parents were unaffected These reports ındicate that there ıs a genetic factor ın the aetiology of this condition and the majority of cases are ınherited as autosomal dominant although other mode of ınheritance could not be ruled out

SUMMARY AND CONCLUSION

Cases of osteogenesis ımperfecta congenita ın one of twins are reported This condition ıs uncommon ın single births and more so among twin deliveries It ıs ınvariably fatal and this will partially explain ıts rarity as many are still born or die shortly after It ıs also possible that the ıncidence of the disease will vary from one geographical area to another depending on the frequency of the particular gene ın the population A more detailed family studies and ınvestigation of new cases ın twins may give more ınformation on the question of ınheritance ın the African population

REFERENCES

1 Awwad S & Reda M Osteogenesis imperfecta *Arch Pediat 77* 280 1960
2 Chawla S Intrauterine osteogenesis ımperfecta ın four sıblings *Brit Med J I 99 1964*
3 Freda V J Vosburgh G J & Diliberti C Osteogenesis ımperfecta congenita a presentation of 16 cases and review of the literature *Obst Gynaec 18* 535 1961
4 Goldfarb A A & Ford D Osteogenesis imperfecta congenita ın consecutive siblings *J Pediat 44* 264 1954
5 Heys F M Blattner R J & Robinson H B G Osteogenesis ımperfecta and odontogenesis ımperfecta clinical and genetic aspects ın eighteen families *J Pediat 56* 234 1960
6 Sarma V A case of intrauterine osteogenesis ımperfecta *Brit Med J II* 1856 1960
7 Seedorff K S *Osteogenesis imperfecta A study of clinical features and heredity Based on 5 Danish families comprising 180 affected persons* Universitetsforlaget Arhus 1949 vol 20
8 Smårs G Osteogenesis ımperfecta ın Sweden Clinical genetic epidemiological and socio medical aspects Scandinavian Univ Books Stockholm 1961
9 Vrolik W cited by J Bell *Blue sclerotics and fragility of bone* ın *Treasury of Human Inheritance* 2 III Francis Galton Laboratory for National Eugenics University of London 1928
10 Zeitoun M M Ibrahim A H & Kassem A S Osteogenesis ımperfecta congenita ın dizygotic twins *Arch Dis Child 38* 289 1963

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Key words Osteogenesis ımperfecta congenita twins inheritance

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CASE REPORT

GROWTH HORMONE RESPONSE TO INSULIN INDUCED HYPOGLYCEMIA IN A BOY WITH DIABETES INSIPIDUS AND SHORT STATURE BEFORE AND AFTER TREATMENT WITH VASOPRESSIN

P OLIN

From the Department of Paediatrics Kronprinsessan Lovisas Barnsjukhus Karolinska Institutet Stockholm Sweden

The problems of evaluating the plasma growth hormone (GH) response to insulin induced hypoglycemia in children of short stature are now well recognized (7) The patient with short stature and diabetes insipidus that is presented here adds some pertinent data in this context.

CASE REPORT

A 15 58 year old boy was referred from the school physician because of stunted growth since the age of 12 years His father and elder brother 22 years old were 172 cm tall and his mother was 168 cm No endocrine disorders were known in the non-consanguineous family During the last four years preceding the admittance to the hospital the patient had been easily tired but managed very well at school His skin became dry He did not sweat He was more sensitive to cold temperature He had been drinking excessively and voided several times every night. The analyses of the growth data obtained from the school records and at the first visit revealed a marked retardation in development

As shown in Fig 1 his height was normal around −1 S D to the age of 11 6 years The height increment was 4 5 to 6 cm/year During the next 3 4 years the increment was only 1 6 to 1 cm/year A slightly increased growth rate of 3 1 cm/year was noted during half a year preceding the admittance

Physical examination

At the chronological age of 15.58 his height was 146 cm (−3 S D) span 146 cm lower segment 74 cm Weight 35 5 kg (average for height) There was a slight development of the secondary sex characteristics Penis and scrotum was not stimulated Pubic hair was sparse grade II according to Greulich (1) The testes were descended and each were 25 × 15 mm The skin was dry and mottled and the subcutaneous tissue was increased Eye movements and vision were normal The ankle jerk relaxation time was not prolonged Deep tendon reflexes were present

Laboratory data

Routine hematology was normal Some data regarding the pituitary function are given in Table 1 The results were consistent with an antidiuretic hormone (ADH) deficiency The thyroid function tests were essentially normal although a mild deficiency of thyroid stimulating hormone (TSH) could not be excluded The normal response of urinary 17 OH-corticosteroids to metyrapone indicated a normal adrenocorticotrophin (ACTH) adrenal function The urinary gonadotrophins were low but the boy was in early puberty judged by clinical criteria such as the size of the testes the presence of pubic hair and the slight increase in growth rate Radiological examinations of the skull and sella were normal An EEG and a pneumoencephalogram including repeated tracings of the visual field were normal The skeletal age was 12 years according to the atlas of Greulich & Pyle (3)

Clinical course

A diagnosis of combined ADH GH and possibly TSH deficiency was suspected (Data on plasma GH is presented under Special studies) Treatment with thyroxin and a nasal preparation of vasopressin was given for six months As shown in Fig 1 the growth rate increased When human growth hormone (gift from Dr Roos Uppsala Sweden) was added to the

four affected siblings—3 males and 1 female Remaining 3 siblings as well as the parents were unaffected These reports indicate that there is a genetic factor in the aetiology of this condition and the majority of cases are inherited as autosomal dominant although other mode of inheritance could not be ruled out

SUMMARY AND CONCLUSION

Cases of osteogenesis imperfecta congenita in one of twins are reported This condition is uncommon in single births and more so among twin deliveries It is invariably fatal and this will partially explain its rarity as many are still born or die shortly after It is also possible that the incidence of the disease will vary from one geographical area to another depending on the frequency of the particular gene in the population A more detailed family studies and investigation of new cases in twins may give more information on the question of inheritance in the African population

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1 Awwaad S & Reda M Osteogenesis imperfecta *Arch Pediat 77 280 1960*
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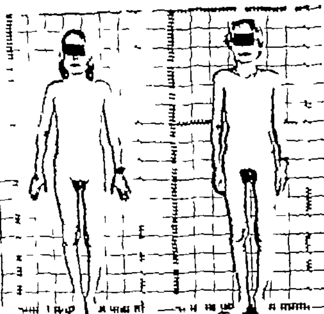


Fig 2 The patient at the first admittance when he was 15 85 years old (left) The skin was pale and dry Slight pubertal development is evident The appearance of the patient one year later at the age of 16 85 years (right) when he had been on treatment with thyroxine and growth hormone for 5 months. The subcutaneous tissue is markedly reduced The growth increment 5 cm/year The pubertal development was more advanced

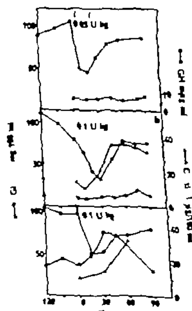


Fig 3 I v insulin tolerance tests (*a*) At the age of 15 83 no therapy was given The plasma GH response to hypoglycemia is blunted (*b*) After treatment with thyroxine 0   mg/day for three months the plasma GH response is inadequate (*c*) At the age of 16 85 when pitressin tannate in oil had been given 5 U im daily for ten days The plasma growth hormone response is normalized

growth hormone was discontinued 20 days before this study The boy was on a nitrogen constant diet for 5 days before the test As shown in the figure the basal plasma growth hormone levels were higher than in the earlier tests and the response to hypoglycemia was marked Plasma cortisol also increased adequately

COMMENTS

The striking feature in this patient is that the treatment with vasopressin normalized a previously abnormal growth hormone response to insulin induced hypoglycemia Untreated or poorly controlled diabetes insipidus due to vasopressin insufficiency in childhood is suggested to cause an inadequate caloric intake followed by hypocaloric dwarfism (6) It is not clear whether the so called hypocaloric dwarfism is caused by a general depletion of nutrients such as protein to the growing tissues or if there is insufficient synthesis or release of growth hormone The data from this patient favours that vasopressin is necessary to allow an adequate release of growth hormone The absence of other signs of malnutrition such as anemia low serum protein and depletion of body fat makes an inhibition of growth hor

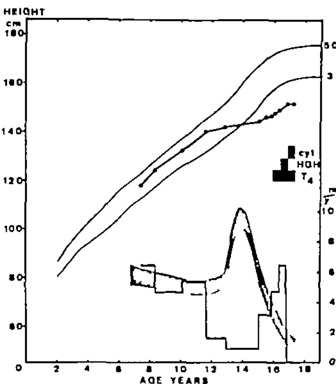


Fig 1 Growth chart The height in centimeter is plotted against chronological age in the upper part of the figure In the lower part the height velocity is given in cm/year The growth data were obtained from the school record up to the age of 15 8 Thereafter the height was measured in the morning on visits to the hospital Each of these measurements represent the mean of two readings The data are plotted on the growth chart of Tanner *et al* (5) The 50th and 3rd percentile for height is given The shaded area represents the growth velocity curve for longitudinally followed boys of average height according to Tanner *et al* (5) The black bar indicates *periods of therapy CYT = Cytostatic treatment with* vincoblastine i v 0 15 mg/kg per week and prednisolone 5 mg × 4 HGH = Human growth hormone 2 mg twice weekly i m T = thyroxine 0 2 mg daily

regime for another period of 5 months the growth increment was only slightly more augmented

The patient was readmitted at the age of 16 85 years to evaluate the preliminary diagnosis His height was 151 5 cm weight 34 6 kg The subcutaneous fat was markedly reduced The skin was normal The testes were 35 × 20 mm resp 30 × 20 mm Pubic hair grade III and axillary hair grade II It was realized that the boy had only sporadically utilized the nasal preparation of vasopressin Adequate substitution therapy with pitressin tannate in oil was given and plasma growth hormone was again studied as described below Before any further change in the therapy was undertaken the boy developed symptoms of a cervical rizopathia A radiological examination showed *multiple destructions* of the fifth cervical vertebra and multiple infiltrates in both lungs No other skeletal defect was observed The diagnosis of Hand–Schuller–Christian's disease was established by cytological examination of an aspiration biopsy from the destructed vertebra (performed by Dr S Franzen Stockholm) The patient did not grow at all during the combined treatment with vincoblastine and corticosteroids that followed (Fig 1)

SPECIAL STUDIES

Intravenous insulin tolerance tests were performed after fasting over night when the patient was still in bed A catheter was inserted into a cubital vein two hours before the insulin was injected The catheter was kept open throughout the test by a slow infusion of normal saline All blood samples were drawn from the catheter Plasma growth hormone was determined by a double antibody radioimmunological assay (2) plasma cortisol was determined fluorometrically and blood glucose by a glucose oxidase method The insulin dose was 0 05 U/kg body weight in the first test and in the following tests 0 1 U/kg In all tests the minimum blood sugar level was less than 50 % of the fasting level The first test Fig 3 *a*, was performed at the age of 15 85 years before any treatment was given The plasma growth hormone level did not change during the test The second test Fig 3 *b* was performed when the patient was on thyroxine 0 2 mg daily since three months Plasma growth hormone did not change but for one value of 9 8 mμg/ml at 75 min after the insulin injection Plasma cortisol increased markedly The third test Fig 3 *c* was performed at the age of 16 85 when the patient had received pitressin tannate in oil 5 U i m daily for ten days and thyroxine 0 2 mg daily for one year Treatment with human

Table 1 *Laboratory data regarding the function of the pituitary gland*

Urinary 17 hydroxycorticosteroids mg/24 hours	18
After metyrapone 500 mg × 4	51
PBI μg/100 ml	5 5–6 0
T_3 resin uptake (normal range 85–125)	83
^{131}I uptake after 24 hours	20
After thyrotropin 3 U s.p i m	51
Urinary osmolality mOsmol/kg	130–310
Urinary volume *liters/24 h*	5–7
Urinary gonadotrophins mouse units	<6 5

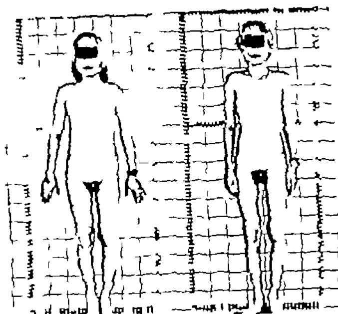


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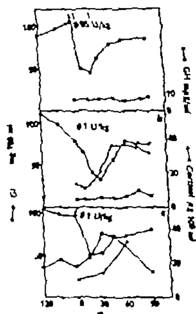


Fig 3 I.v. insulin tolerance tests (a) At the age of 15.85 no therapy was given The plasma GH response to hypoglycemia is blunted (b) After treatment with thyroxine 0.2 mg/day for three months the plasma GH response is inadequate (c) At the age of 16.85 when pitressin tannate in oil had been given 5 U i.m. daily for ten days The plasma growth hormone response is normalized

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mone synthesis improbable although this mechanism cannot be excluded The risks in using a nasal preparation of vasopressin to control diabetes insipidus in childhood (6) was unfortunately confirmed Blunted plasma growth hormone responses to insulin induced hypoglycemia or arginine infusion in patients with diabetes insipidus have been reported by Landon *et al* 1966 (4) and Yuolton *et al* 1969 (7) The authors do *not state if adequate substitution* therapy with ADH was given The data from this patient again demonstrated the fallacy of establishing a diagnosis of growth hormone deficiency on the basis of an absent growth hormone response to insulin induced hypoglycemia before other interferring factors are excluded Hypothyroidism, cortison treatment and malnutrition among other circumstances may blunt the growth hormone response To this list uncompensated diabetes *insipidus should be added*

SUMMARY

A 16-year old boy with Hand–Schuller–Christian s disease with diabetes insipidus and short stature is presented The plasma growth hormone response to insulin induced hypoglycemia was reduced at two occasions but was normalized after adequate substitution with vasopressin The finding is discussed in connection to the pathogenesis of growth retardation in patients with diabetes insipidus

ACKNOWLEDGEMENT

The radioimmunoassay of growth hormone was kindly performed by Dr E Cerasi Stockholm

REFERENCES

1 Anderssen E Skeletal maturation of danish school children in relation to height sexual development and social conditions *Acta Paediat Scand Suppl 185* 32 1968
2 Cerasi E Della Casa L, Luft R & Roovete A Determination of human growth hormone (HGH) in plasma by a double antibody radioimmunoassay *Acta Endocr 53* 101 1966
3 Greulich W W & Pyle I S *Radiographic atlas of skeletal development of the hand and wrist* Stanford California Stanford University Press, 1959
4 Landon J Greenwood F C Stampand T C G & Wynn W The plasma sugar free fatty acids cortisol and growth hormone response to insulin and the comparison of this procedure with other tests of pituitary and adrenal function II In patients with hypothalamic or pituitary dysfunction or anorexia nervosa *J Clin Invest 45* 437 1966
5 Tanner J M Whitehouse R M & Takaishi M Standards from birth to maturity for height, weight height velocity and weight velocity *Arch Dis Child 41* 454 1966
6 Vest, M Talbot N B & Crawford J D Hypocaloric dwarfism and hydronephrosis in diabetes insipidus *Am J Dis Child 105* 175 1963
7 Youlton R Kaplan S L & Grumbach M M Growth and growth hormone IV Limitations of the growth hormone response to insulin and arginine and of the immunoreactive insulin response to arginine in the assessment of growth hormone deficiency in children *Pediatrics 43* 989 1969

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

THE DANISH PAEDIATRIC SOCIETY

Meeting April 9 1969

The future of paediatrics in Denmark Discussion

Meeting May 28 1969

H Kreutzfeldt *Information concerning family advisory activities of the services for children and young people*

K Wilken Jensen *Allergic infantile alimentary purpura*

K Wilken Jensen & J C Melchior *Allergy to foodstuffs and neurological symptoms*

J Møller *Haemolytic uraemic syndrome*

A report is given of a syndrome occurring with increasing frequency Briefly the syndrome consists of an acute condition with development of haemolytic anaemia and uraemia accompanied by thrombocytopenia and a great variety of functional disturbances in other organs including the brain The syndrome has been described exclusively in children but there appears to be a certain justification for regarding it as a variant of Moschowitz syndrome or thrombotic thrombocytopenic purpura which has been described predominantly in adults

A fatal case in a boy aged eight years is described Treatment with dialysis blood transfusions glucocorticoids and late after the commencement of the illness with heparin was without effect

Very little is known of the cause of the condition It is occasionally observed in siblings although this is rare and geographic accumulation is recognized Some authors have observed the occurrence of an ultrafiltrable agent and an increase of antibodies against this substance

All of the symptoms appear to be explained by acute intra vascular coagulation which may be demonstrated inter alia by renal biopsy in the acute phase of the disease The process takes place mainly in the capillaries and the symptoms can be explained by the formation of microinfarcts The thrombocytopenia is explained by absorption of the thrombocytes in the microthrombi Precipitated fibrin and possibly damaged vascular endothelium are considered to provoke the accompanying haemolysis

Treatment is symptomatic and consists of dialysis transfusions and if necessary antiepileptic and blood pressure lowering therapy Most important is the treatment with heparin which has frequently resulted in dramatic improvement if undertaken at an early phase of the disease Glucocorticoids should probably be employed with care as these substances are known to facilitate provocation of the Schwartzmann phenomenon There is a surprising similarity between haemolytic uraemic disease and this purely experimental phenomenon The syndrome should probably be regarded as a localized Schwartzmann phenomenon

mone synthesis improbable although this mechanism cannot be excluded The risks in using a nasal preparation of vasopressin to control diabetes insipidus in childhood (6) was unfortunately confirmed Blunted plasma growth hormone responses to insulin induced hypoglycemia or arginine infusion in patients with diabetes insipidus have been reported by Landon *et al* 1966 (4) and Yuolton *et al* 1969 (7) The authors do not state if adequate substitution therapy with ADH was given The data from this patient again demonstrated the fallacy of establishing a diagnosis of growth hormone deficiency on the basis of an absent growth hormone response to insulin induced hypoglycemia before other interferring factors are excluded Hypothyroidism cortison treatment and malnutrition among other circumstances may blunt the growth hormone response To this list uncompensated diabetes insipidus should be added

SUMMARY

A 16 year-old boy with Hand–Schuller–Christian s disease with diabetes insipidus and short stature is presented The plasma growth hormone response to insulin induced hypoglycemia was reduced at two occasions but was normalized after adequate substitution with vasopressin The finding is discussed in connection to the pathogenesis of growth retardation in patients with diabetes insipidus

ACKNOWLEDGEMENT

The radioimmunoassay of growth hormone was kindly performed by Dr E Cerasi Stockholm

REFERENCES

1 Andersen E Skeletal maturation of danish school children in relation to height, sexual development and social conditions *Acta Paediat Scand* Suppl *185* 32 1968
2 Cerasi E Della Casa L Luft R & Roovete A Determination of human growth hormone (HGH) in plasma by a double antibody radioimmunoassay *Acta Endocr 53* 101 1966
3 Greulich W W & Pyle I S *Radiographic atlas of skeletal development of the hand and wrist* Stanford California Stanford University Press, 1959
4 Landon J Greenwood F C Stampand T C G & Wynn W The plasma sugar free fatty acids cortisol and growth hormone response to insulin and the comparison of this procedure with other tests of pituitary and adrenal function II In patients with hypothalamic or pituitary dysfunction or anorexia nervosa *J Clin Invest 45* 437 1966
5 Tanner J M Whitehouse R M & Takaishi M Standards from birth to maturity for height weight height velocity and weight velocity *Arch Dis Child 41* 454 1966
6 Vest, M Talbot N B & Crawford J D Hypocaloric dwarfism and hydronephrosis in diabetes insipidus *Am J Dis Child 105* 175 1963
7 Youlton R Kaplan S L & Grumbach M M Growth and growth hormone IV Limitations of the growth hormone response to insulin and arginine and of the immunoreactive insulin response to arginine in the assessment of growth hormone deficiency in children *Pediatrics 43* 989 1969

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Key words Diabetes insipidus Hand–Schuller–Christian s disease growth hormone response vasopressin treatment short stature

Discussion

I Vesterdal Acidosis has been described in premature infants fed on acidified milk preparations Feeding with cows milk has been employed since Arild's time without deficiency symptoms being described

N J Brandt What is the vitamin E content?

J Clausen The vitamin E content is 20% lower in skim milk preparations than in the other preparations

E Nathan Are these quantities in weight per cent?

J Clausen The determinations are carried out by gas chromatography and thus express the content by weight

J Clausen & B Friis Hansen *Lipoproteins in newly born infants*

Investigations of lipid metabolism in adults in recent years has revealed that the lipid absorbed from the food after transport to the liver or directly in the intestinal walls is taken up or combined partly in the chylomicron fraction and partly in the lipoprotein fractions which migrate electrophoretically in the α and β zones (cf review by Burstein, 1961) Further α-lipoprotein occurs in serum and does not possess the abovementioned transport function β lipoprotein consists immunologically of at least two fractions with partial immunological identity (viz α 2 and β lipoproteins) (3) By means of electrophoretic separation in albumin-containing agarose the β lipoprotein can be separated from the α 2 lipoprotein which is also termed pre β lipoprotein which is of diagnostic significance for subdivision of anomalies of lipoproteins (2)

At birth, the newly born organism undergoes transition from parenteral to oral feeding In agreement with the parenteral feeding of the foetus the authors found on investigation of cord blood that newly born infants lack or have a very low content of β and α 2 lipoprotein, the dominating lipid fraction in cord blood being a fraction with an electrophoretic migration resembling α 1 lipoprotein

By means of electrophoresis in albumin-containing agarose it was found, as a function of the time which had elapsed since birth, that the quantity of β lipoprotein rose in the course of the first two weeks By means of quantitative radial immunodiffusion, it was demonstrated that the adult β lipoprotein level is not attained until the second or third week of life at the earliest and that this is influenced by individual differences

Continued investigations will reveal whether retarded development of α and β lipoproteins are connected with the well being of infants

REFERENCES

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Discussion

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J Vesterdal Is it not possible that transport lipoproteins are present in newly born infants but do not become manifest until lipids are present?

J Clausen In hyperlipaemia the protein fraction varies but not the quantity of lipids which appears to contradict the above presumption

N Hobolth How were these infants fed?

J Clausen As a rule with breast milk

P Kraulukoff Are β and α 2 lipoproteins equally suitable for lipid transport?

J Clausen The β lipoproteins are the most important and α 2 lipoproteins only constitute 10 per cent of the total fraction in normal adults

P Paerregaard

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Meeting September 25 1969

Gerald Gaull *Paediatric research and mental retardation*

Meeting October 8 1969

N J Brandt & J Philip *Hereditary Cri du Chat Syndrome*

A woman aged 33 years gave birth to an infant with typical Cri du Chat Syndrome As she had previously had a spontaneous abortion, a family investigation was undertaken The only abnormal finding in the phenotypically normal mother was a mosaic of normal cells and cells with deletion of the short arm of chromosome no 5 The mother became pregnant again and therapeutic abortion was undertaken The foetus had the same chromosome abnormality as is characteristic for the Cri du Chat Syndrome This is the first description of a mother with mosaicism for deletion of the short arm of chromosome no 5 giving birth to offspring with the Cri du Chat Syndrome

This report emphasizes the significance of undertaking chromosome investigation in the parents of all children with chromosome anomalies *as it appears that parents such as these* may be mosaics to a greater extent than previously presumed (To be published in *J Med Genet*)

Discussion

Lise Wagner What was the mother's voice like?

N J Brandt Normal

H Dyggve How many cases are there in Denmark?

J Philip This is not known but Niebuhr has hitherto found 20 among mentally retarded patients

B Friis Hansen & J Clausen *Lipid metabolism in mothers and newly born infants*

On account of the increasing difficulty in obtaining breast milk in recent years *it has* become increasingly necessary to employ breast milk substitutes and development has tend towards provision of humanized milk In d ing this the number of unsaturated fatty ac has been increased by removing milk fat a replacing this by vegetable oils which are r in poly unsaturated fatty acids This togeth with the *interest in the etiology of the de*generative conditions of the central nervous sy tem and the present investigations have t*gether aroused interest in this department* the relationship between the serum lipids mothers and newly born infants and alter tions in the serum lipid levels in infants r ceiving different diets The results are expre sed as a percentage of the total fatty acid co tent following fat extraction in milk or seru and gas chromatographic investigation of th fat

The linoleic acid content of breast milk approximately 9–12 • In cows milk on th other hand the content is approximately 3 4% in a half-skimmed milk preparation (El don) it is 7% and in a fat substituted mi preparation (Semper) it is about 18–26 I cord blood the linoleic acid content is approx imately 13° In contrast the content of ara chidic acid in maternal blood is 4–5% com pared with 8% in cord blood After the elaps of a week the content of linoleic acid in brea fed infants increases to 10–15• while in in fants fed on preparation Semper this increase to the double viz approximately 30% Th importance of the breast milk substitutes con taining a well balanced mixture of unsaturate fatty acids is emphasized because too high content of an individual fraction can inhibi the metabolism of the other fractions Further administration of large quantities of linolei acid can result in a relative deficiency of vita min E

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P Paerregaard

BOOK REVIEWS

V Dubowitz *The floppy infant* Clinics in Developmental Medicine No 31 W Heinemann Medical Books Ltd London 1969 109 pp US $6 00

On 100 pages Victor Dubowitz reviews the causes and the management of muscular hypotonia in infancy His description is clear and concise and well illustrated with pictures of children with muscular hypotonia of various causes and with the histopathological findings in the muscles particularly in the rare forms of congenital myopathy

The author uses the presence or absence of true weakness in the hypotonic infant as the first clue to a differential diagnosis The conditions characterized by muscular weakness are surveyed in chapter III The quantitative dominance of progressive spinal muscular atrophy is stressed and the various types of rare and recently described congenital myopathies are well outlined and illustrated with personal cases

In the next chapter conditions causing muscular hypotonia without weakness are reviewed The emphasis is here on disorders associated with mental retardation certainly the most common cause of muscular hypotonia in early infancy Examinations useful in establishing a differential diagnosis are briefly outlined

The approach recommended by the author always first to decide whether or not true weakness is present and then proceed along one of 2 different lines is sensible and rewarding in most cases However the author has not entirely followed his own outline Thus conditions included in chapter 3 seem to have the anatomical localisation within the motor unit in common rather than the presence of the clinical sign weakness and in chapter 4 conditions with definite weakness but without evidence of involvement of the motor unit are also described A severe perinatal lesion of the cervical cord must cause both hypotonia and weakness and so does Prader Willi s syndrome both are described in chapter 4 It is true that the profound hypotonia seen in Prader Willi s syndrome is out of proportion to the moderate weakness this is however often so severe that these patients are misdiagnosed as cases of muscular dystrophy A line about Prader Willi s syndrome in chapter 3 had therefore been helpful to the clinician trying to diagnose a given case of muscular weakness and hypotonia

In chapter 5 the diagnostic procedures are described and the conditions outlined in which certain procedures are of special help The great value of a muscular biopsy is stressed somewhat at the expense of electromyography which should have been mentioned as a necessary procedure to establish or exclude myotonic dystrophy in a young infant Th difficulties stressed by the author in interpreting th electromyogram of an infant may decrease with th examiner s increasing experience of the procedure i this age group It adds greatly to the value of th book that the author also gives a description of when and how to take a muscular biopsy and how t handle it before it reaches the pathologist As th author recommends the quadriceps muscle for rou tine biopsy it should perhaps be pointed out tha intramuscular injections given in the muscle interfere with the histological picture to at least the same ex tent as an EMG needle

Doctor Dubowitz s approach to the problem of infantile muscular hypotonia is sensible and thor oughly enjoyable for the clinician The patient pre sents himself to the doctor with a series of symptoms not with a diagnosis and all clinical teaching ought to start with symptoms and symptoms and signs lead up to a diagnosis This book is obviously written by an excellent clinician and teacher of clinical medi cine and it can be highly recommended to all physi cians dealing with young children

Ingrid Gamstorp

D G Evans (ed) *Immunization against infectious diseases* British Medical Bulletin *25* No 2 London 1969 £2

Several British authorities contribute to the information given in this journal After an introductory ar ticle on world problems in viral vaccines and a survey of the search for protective antigens more specific information is given about the different viral and bacterial vaccines

Some authors have pointed out that the risks of damaging complications from smallpox vaccination are so great that it should not be justified in countries free from infection However the importation of smallpox into several countries in Europe recently makes thorough programs of vaccination desirable Use of antivaccinia gammaglobulin is indicated in cases of vaccinia gangrenosum and as a prophylactic measure in higher risk groups *It also seems to be* successful in preventing postvaccinal encephalitis

The choice of killed or attenuated polio vaccine is still open to some controversy *In most European* countries and in the United States the attenuated vaccine is the most generally accepted both for rou tine prophylaxis and epidemic control However there are disadvantages such as inhibition in the gut

by interfering viruses and risk of causing paralysis Swedish investigators have shown that killed vaccines produce both short and long term immunity which serologically measured is as good as that produced by attenuated vaccines

Clinical trials have now been carried out with measles vaccines and approximately 35 million doses of an attenuated vaccine have been used Reports of severe illnesses associated with vaccination have been few The degree of protectiveness is very high

Live attenuated rubella vaccines have been administered to several thousand susceptible individuals mainly children Mild reactions consisting of upper respiratory symptoms lymphadenopathy and involvement of joints have been reported

No mention is made of vaccinations against mumps although some clinical trials have already been carried out

At the moment vaccines against upper respiratory infections make no significant contribution to public health. They had to contain so many numerous components perhaps more than 100 that it is not surprising that preparation of such vaccines has not yet been reported

Evidence is now accumulating that BCG is of value not only in protecting against tuberculosis but against other mycobacterial infections as well

Untoward reactions to combined vaccines containing pertussis vaccine have been attributed to the pertussis component It is stated that the reaction rate is significantly lower in a series in which a triple vaccine with an aluminium adjuvant is used No comments are made on complications from the nervous system

Concerning typhoid paratyphoid vaccines it is said that certain vaccines containing Salm typhi are effective in the field The value of the paratyphoid components is doubtful This is in accordance with clinical experience from many other places

This journal also contains instructive articles on rabies vaccine arbovirus vaccines rickettsial vaccines diphtheria and tetanus toxoids cholera vaccines protozoal vaccines and the use of human immunoglobulin It is strongly recommended to all those interested in the prophylaxis of infectious diseases and in public health

Ulf Bergdahl

D J Beintema *A neurological study of newborn infants* Clinics in developmental medicine No 28 W Heinemann Medical Books Ltd London 1968 178 pp illus 37

In sample of 49 full term infants neurological examinations were made every day for the first 9 days after birth Infants with severe abnormalities or severe illness were eliminated from the study The neurological examination used is the one previously developed in Groningen (Prechtl & Beintema 1964) Each item was scored on a quantitative scale and all data were statistically analysed The purpose of this investigation was to study the consistencies and inconsistencies of the neonatal neurological signs Three basic questions were asked

1 *What standardisation procedures should the examiner introduce in order to get reliable information from the neurological examination?* Ambient temperature clothing and hunger which influence the nervous function of newborn infants are very well controlled in this study The state" (of sleep and wakefulness) of the infant was studied before and during the neurological examination The time course and alterability of the state by handling the baby were investigated One of the conclusions is that the optimal state for many of the neurological tests is less often present during the first days after birth.

2 *What is the developmental course of neurological signs during the first days of life?* The data regarding age dependance showed three kinds of development of the neurological signs (*a*) signs which were weaker or more difficult to elicit on the 1st–4th days than on the 5th–9th (e g responses in which motility is involved) (*b*) a small group opposite to (*a*) i e signs which were weaker on the 5th–9th days than on the 1st–4th (e g resistance to passive movements recoil of the forearm and elbow) (*c*) signs which remain constant throughout the neonatal period (e g abdominal skin reflexes blink reflexes tendon reflexes) Another important point is the persistence of a characteristic behavior pattern It was concluded that from about the 4th day the findings were representative for the rest of the neonatal period which means that a neurological examination carried out on the first three days is less valid than those in later days

3 *Do obstetrical and postnatal conditions affect the developmental course and the consistency of neurological signs throughout the neonatal period?* Pre and perinatal obstetrical data were compared with the daily scores of neurological items In addition a high risk and a low risk group were compared Infants with the umbilical cord around the neck and apnoea at birth often got an apathy syndrome on the first 4 days The difference found between the low and high risk groups was that babies of the latter group had lower resistance to passive movements on the first day and lower intensities for some other tests It was concluded that infants who had suffered from perinatal complications often had a prolonged adaptation period—a general depression of the nervous system on the first few days Most of the infants who had a moderate resistance to passive movements on the first day and be sme hypotonic later on appeared to be jaundiced and/or had gastro-intestinal signs later

Prof Heinz Prechtl points out in the preface of this book that the knowledge of the CNS of the neonate is still scanty The reader will agree that Beintema s study of the neurology of newborn infants is an excellent attempt to fill this gap The results of this thorough critical and interesting study which have been presented as a thesis are of great importance for the evaluation of neurological examina

tions of neonates Thus the book can be warmly recommended to all paediatricians or neurologists concerned with such examinations

Jan Ekholm

J G Howells (ed) *Modern perspectives in international child psychiatry* Oliver & Boyd Edinburgh 1969 878 pp £8 8s

Perhaps child psychiatry is the speciality that has had most difficulties in finding its identity among medical professions In his introduction to Modern Perspectives in International Child Psychiatry Leo Kanner says *In the light of present-day usage it* sounds incredible that the term child psychiatry itself had not acquired formal citizenship in the realm of professional or any other parlance until a little less than three decades ago The reason for this is that child psychiatry more than other medical disciplines is founded on the behavioural sciences Child psychiatry is a fusion of what used to be a collection of more or less loosely scattered segments Its roots are not only in psychiatry proper and in pediatrics but also in education psychology criminology and sociology For the medical student with his traditional training in the natural sciences there may be a certain difficulty to find his professional ego in a discipline where so many non medical sciences also dominate the scene But on the other hand even if it is difficult to define child psychiatry as a medical profession it surely exists as a *functional* concept and as a result of many pressing needs to solve mental problems in childhood

This new edition of Modern Perspectives may be seen as an illustration of Kanner's notion that child psychiatry is a result of the convergence of many interests The authors represent very different approaches and professions Contributions come from Canada *France Germany Israel Italy Japan Sweden* The United Kingdom The United States, and The USRR This work ought to be of great interest not only to child psychiatrists but also to those working *in related professions and specialities—medical* or non medical There are twenty six different articles treating subjects such as adverse perinatal factors (Stott) separation and deprivation (Howells) the management of ulcerative colitis (Prugh & Jordan) childhood psychosis (Bender) residential care of children (Ahnsjo) and the strategy of follow up studies (Robins & O Neal) to take only a few Together with the first edition of Modern Perspectives in Child Psychiatry (2nd ed 1967) this book is a very valuable source of knowledge in child psychiatry and perhaps the most comprehensive available at present It surely gives the impression that child psychiatry has an identity of its own

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ANNOUNCEMENT

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Acta Pædiat Scand 59 353–362 1970

INTENSIVE CARE OF SMALL PREMATURE INFANTS

I Clinical findings and results of treatment

EERO K VAPAAVUORI and NIELS C R RÄIHÄ

From the Department of Paediatrics University Central Hospital Helsinki Finland

During recent years there has been a growing concern over the intensive care of newborn infants suffering from respiratory insufficiency Most reports however include only a few cases of small premature infants (1 6 23 24) who are a high risk group with high mortality and high incidence of respiratory failure requiring assisted ventilation On the other hand follow up studies reported by a number of workers (8 10 13) indicate that the incidence of severe brain damage in these infants is very high The deficiency of detailed clinical data during the neonatal period however makes it difficult to draw any conclusions about the possible etiologic factors responsible for the neurologic and mental abnormalities indicative of permanent brain injury

Thus we found it important to conduct a prospective study on the intensive care of premature infants with birth weights between 850 and 1250 g and to discuss the correlation between clinical findings response to the treatment and outcome

MATERIAL AND METHODS

The study comprises 49 premature infants weighing from 850 to 1250 g at birth admitted to the Neonatal Unit of the University Children's Hospital Helsinki during the two-year period 1966–1967 All infants with major congenital malformations were excluded

Supported in part by grants from the Foundation for Pediatric Research in Finland and the Association for the Aid of Crippled Children New York.

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All infants were nursed in incubators with a high environmental humidity and maintaining skin temperature at 36 5 C As soon as possible after admission, one of the umbilical arteries was catheterized and following a 10 minute period of breathing 90–100% oxygen arterial pH Pco and Po were determined (19) The umbilical vein was also catheterized to begin intravenous early feeding using 10 glucose solution (50 ml per kg per 24 hrs) To prevent infection antibiotics usually cloxacillin (50 mg per kg per 24 hrs) and colimycin (50 000 units per kg per 24 hrs) were given intramuscularly in two divided doses To correct acidosis rapid sodium bicarbonate infusions were given according to the acid base status A chest roentgenogram was obtained if possible during the first 24 hours of life

Apnea which could not be overcome by peripheral stimulation was treated with endotracheal intubation and short (manual) ventilation Accumulation of CO (over 80 mm of mercury) and falling Po in arterial blood (below 50) were also indications for starting intermittent positive pressure respiration (IPPR) A positive pressure respirator (BENNETT PR 1 or PR) and occasionally manual ventilation was used The inspired oxygen concentration was adjusted to maintain the arterial Po between 50 and 100 mm of mercury

CLINICAL DEFINITIONS

Respiratory distress (RD) All patients were divided into three categories according to the severity of their respiratory symptoms during the first 12 to 24 hours of life On admission a careful physical examination was made including an evaluation of the following symptoms 1) cyanosis 2) expiratory grunting 3) intercostal and substernal retractions 4) abnormal respiratory rate and 5) apneic attacks Severe birth asphyxia (5 minute Apgar score 0-5) associated with apnea which did not respond to resuscitation or a rapidly progressing respiratory insufficiency including all the symptoms indicated above was defined as severe respiratory distress The occur

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J G Howells (ed) *Modern perspectives in international child psychiatry* Oliver & Boyd Edinburgh 1969 878 pp £8 8s

Perhaps child psychiatry is the specialty that has had most difficulties in finding its identity among medical professions In his introduction to Modern Perspectives in International Child Psychiatry Leo Kanner says In the light of present-day usage it sounds incredible that the term child psychiatry itself had not acquired formal citizenship in the realm of *professional or any other parlance* until a little less than three decades ago The reason for this is that child psychiatry more than other medical disciplines is founded on the behavioural sciences Child psychiatry is a fusion of what used to be a collection of more or less loosely scattered segments Its roots are not only in psychiatry proper and in pediatrics but also in education psychology criminology and sociology For the medical student with his traditional training in the natural sciences there may be a certain difficulty to find his professional ego in a discipline where so many non medical sciences also dominate the scene But on the other hand even if it is difficult to define child psychiatry as a medical *profession it surely exists* as a functional concept and as a result of many pressing needs to solve mental problems in childhood

This new edition of Modern Perspectives may be seen as an illustration of Kanner's notion that child psychiatry is a result of the convergence of many interests The authors represent very different approaches and professions Contributions come from Canada France Germany Israel Italy Japan Sweden The United Kingdom The United States and The USRR This work ought to be of great interest not only to child psychiatrists but also to those working in related professions and specialties—medical or non medical There are twenty six different articles treating subjects such as adverse perinatal factors (Stott) separation and deprivation (Howells) the management of ulcerative colitis (Prugh & Jordan) childhood psychosis (Bender) residential care of children (Ahrnjo) and the strategy of follow up studies (Robins & O Neal) *to take only a few* Together with the first edition of Modern Perspectives in Child Psychiatry (2nd ed 1967) this book is a very valuable source of knowledge in child psychiatry and perhaps the most comprehensive available at present It surely gives the impression that child psychiatry has an identity of its own

Michael Bohman

ANNOUNCEMENT

Under the auspices of the International Pediatric Association the Iranian Society of Pediatrics in collaboration with the Middle Eastern Mediterranean Pediatric Society is organizing in Tehran from Sept *13 16 1970 the VII* Middle Eastern Mediterranean Pediatric Congress For additional information please write Iranian Society of Pediatric P O Box 14/1761 Tehran Iran

diffuse reticulo-granular pattern characteristic of a moderate idiopathic respiratory distress syndrome (IRDS) and grade III (Fig. 1 c) represents the X-ray seen in the most severe cases of IRDS where the configuration of the heart cannot be distinguished from the totally opaque and airless lung fields. The air-filled bronchial tree is visualized as the typical air bronchogram. Aspiration and pneumonia can usually be differentiated from the pulmonary changes indicative of IRDS by the more patchy and lobar infiltrations (Fig. 1 d).

Diagnosis

The clinical diagnosis of IRDS was based on the characteristic respiratory symptoms (expiratory grunting, retractions, cyanosis and high respiratory rate) and on the chest X-ray findings. In most cases of suspected intrauterine pneumonia there was a history of early rupture of the foetal membranes associated with foul-smelling amniotic fluid at birth. In addition, chest X-rays revealed patchy and lobar infiltrations in some of the cases.

A diagnosis of massive intraventricular brain haemorrhage (IVH) was based on a typical course of the illness. Following a comparatively symptomless period, sudden collapse with respiratory and circulatory failure was seen, occasionally associated with convulsions and a bulging fontanel. Further evidence suggesting IVH was a fall in blood hemoglobin values. In one case only was it possible to confirm the diagnosis by demonstrating blood in the spinal fluid.

RESULTS

Clinical findings

The material is summarized in Table 1. Of the total of 49 infants, there were 27 deaths giving an overall mortality rate of 55%. There was no difference between mean birth weights, whereas gestational ages show a difference of 2 weeks between survivors and deaths. Fig. 2 illustrates the correlation between birth weight/gestational age and mortality on the intrauterine growth chart by Backstrom & Kauppinen 1968 (3). Of the infants with gestational ages below 29 weeks, 20 died as compared with only 3 dying of the 12 small-for-date infants (gestational ages over 30 weeks).

Detailed clinical data for the survivors are presented in Table 2. There was only one surviving case defined as belonging *to the group of severe RD*. This infant survived prolonged IPPR begun due to *severe IRDS*. In the group *of moderate RD* there were 6 cases diagnosed

Table 1. *Summary of the material*

Data	Survivals	Deaths
Total number of infants	22	27
Mean birth weight (g)	1090	1090
(range)	(900–1250)	(850–1230)
Mean gest. age (weeks)	29	27
(range)	(24–34)	(24–34)
Complications during pregnancy and delivery	15	22
Sex M/F	12/10	15/12

as having IRDS, of whom 4 showed only transient signs indicating pulmonary hyaline membranes. There were 3 cases suffering from apneic attacks which could not be overcome by short resuscitation and where IPPR was started at the age of 21, 78 and 96 hours respectively. No signs indicating IRDS were seen in these cases. Of the remaining 5 infants, 2 had hypoglycemia and 3 were symptomless. Of the 7 cases suffering from slight or *no* RD, one was treated on IPPR for an apneic attack occurring at the age of 72 hours. There were 2 small-for-dates infants of toxemic mothers and the remaining 4 were symptomless.

Table 3 presents the clinical data for the expired cases treated on IPPR and grouped according to the survivors into 3 categories of

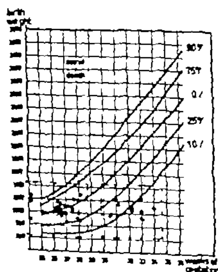


Fig. 2. Correlation between birth weight, gestational age and mortality on the growth chart by Backstrom & Kauppinen 1968 (3).

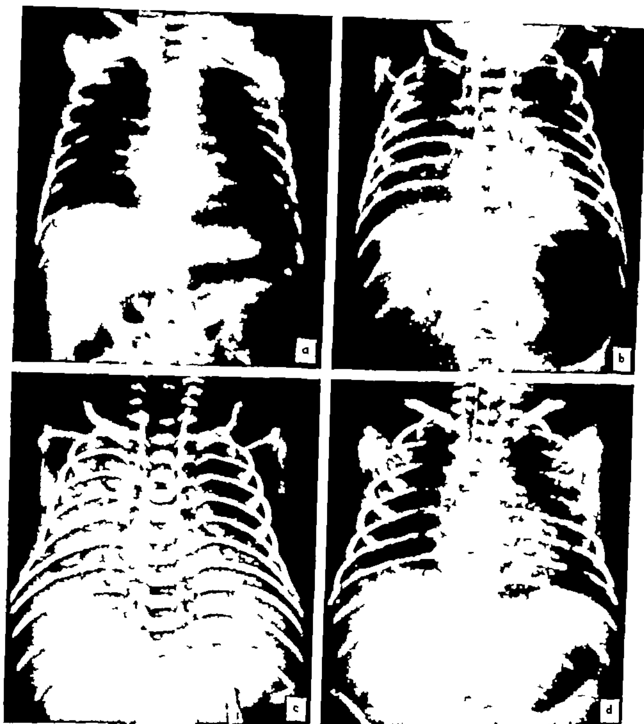


Fig 1 Chest roentgenograms grade I to III (*a–c*) illustrating the reticulo granular density and air bronchogram characteristic of IRDS and patchy an lobar infiltrations indicating pneumonia (*d*)

rence of some of the respiratory symptoms following *slight or moderate birth asphyxia (5 minute Apgar score 4–7)* and a more gradual progression of the re spiratory symptoms was termed *moderate respiratory distress* Finally when there was usually no birth asphyxia (5 minute Apgar score 7 or more) and only transient or mild respiratory symptoms occasionally *followed by an unexpected apneic attack requiring* mechanical ventilation the condition was defined as *slight or no respiratory distress* It must b emphas ized however that in this special weight group som *degree of retraction and irregular breathing is ofte* een in otherwise symptomless surviving infants

Chest roentgenograms

The chest roentgenograms have been divided into three categories according to the degree of reticular *granularity as illustrated in Fig 1 grade I (Fig 1 a)* shows only slightly increased densities and also in clud s normal findings grade II (Fig 1 *b*) shows the

Table 3 *Deaths detailed clinical data grouped according to the three categories of respiratory distress*

(IRDS – idiopathic respiratory distress syndrome IVH – intraventricular brain hemorrhage INF – infection PH – massive pulmonary hemorrhage BPD – bronchopulmonary dysplasia)

Case no	B W (g)	Gest age	Complic factors at birth	Birth asphyxia	Age on admiss hr/min	Onset of IPPR (hr)	Chest X ray	Init art pH	Init art P mm Hg	Diagnosis
I *Severe RD*										
23	1100	34	Caes section	Severe	—/35	At birth	II	7 07	30	IRDS PH
24	970	28	Ablatio plac	Severe	1/—		II	6 99	—	IRDS
25	1000	26	Hydramnion	Severe	1/30		III	7 25	40	IRDS PH
26	11 0	26	Early rupture of the membranes	Severe	—/50		—	6 55	33	INF IVH
27	1080	28	—	Severe	45		I	7 09	—	IVH
28	1050	6	Placenta previa	Moderate	5/—	6	III	7 06	59	IRDS
29	1250	24	Twin birth B	Moderate	4/—	4	III	7 17	78	IRDS
30	1230	28	Twin birth B	No	3/40	4	III	7 25	—	IRDS
31	[illegible]	5	Early rupture of the membranes	Moderate	5/50	6	II	6 85	24	INF
32	1270	31	Twin birth A	No	2/—	14	III	7 06	80	IRDS BPD
33	1000	28	Early rupture of the membranes	No	—/40	1	I	6 96	26	IVH PH
II *Moderate RD*										
34	1200	28	Hydramnion	No	1/—	15	—	7 13	—	IRDS
35	850	77	Twin birth A	No	3/45	125	I	7 12	41	IVH pneumoth
36	1230	33	Breech delivery	Severe	2/45	23	—	7 31	470	IVH INF
37	1100	27	Early rupture of the membranes	No	7/10	29	II	7 07	64	INF IRDS
38	860	24	—	No	1/35	3	I	6 90	124	IRDS
39	1000	26	Caes section	Moderate	1/43	5	II	7 22	8	IRDS PH
40	990		Twin birth B	Moderate	3/18	111	I	7 21	260	IRDS IVH
III *Slight or no RD*										
41	1100	27	—	No	9/30	96	II	7.26	—	Apnea pneumoth
42	1100	9	—	No	1/45	96	II	7.28	360	IVH pneumoth
43	1050	26	Early rupture of the membranes	Moderate	3/—	6	I	7 04	400	IRDS INF
44	9-0	26	Caes section	No	—/20	13	I	7 31	250	Apnea INF (sec)
45	1110	7	Early rupture of the membranes	No	-/30	9	I	7 32	475	INF IVH
46	1000	25	Early rupture of the membranes	No	46/—	59	I	7.29	30	INF
47	1000	27	—	No	—/10	55	I	7 18	510	Apnea INF (sec)
48	1100		Early rupture of the membranes	Moderate	2/—	15	I	7 22	53	INF IRDS
49	[illegible]	?	Breech delivery	Moderate	1/-5	28	—	7.2-	—	IVH

In Table 5 the mean valu s of initial arterial pH P_{CO} and P_O (determined following a 10 minute period of breathing 90–100 oxygen) and th calculated R L shunt percentages (16) are given The most marked differences are seen in pH P_O and R L shunt values in the expired cases of *severe RD* pH values below 7 00 were found *r 4 a*% P_O values were below 100 mm of mercury and the R L shunt percentage (mean) was 50 as compared with 28 in survivors Both pH and P_O were remarkably higher in the other two groups and there was also a striking difference between survivors and deaths As an interesting *finding* there were only 3 surviving infants with initial pH values below 7 20

Response to IPPR

In Fig 3 the response to mechanical ventilation as demonstrated by changes in arterial

Table 2 *Survivors detailed clinical data grouped according to the three categories of respiratory distress*

(IRDS = idiopathic respiratory distress syndrome IVH = intraventricular brain hemorrhage INF = infection PH = massive pulmonary hemorrhage BPD = bronchopulmonary dysplasia RLF = retrolental fibroplasia)

Case no	B W (g)	Gest age	Complic factors at birth	Birth asphyxia	Age on admiss hr/min	Onset of IPPR (hr)	Chest X ray	Init art pH	P mm Hg	Diagnosis
I *Severe RD*										
1	1160	31	Hydramnion breech delivery	Moderate	1/—	32	II	7 21	140	IRDS
II *Moderate RD*										
2	1250	28	Breech delivery	Severe	1/15	—	I	7 26	195	IRDS
3	995	27	Caes section	No	—/30	—	—	7 15	—	IRDS
4	1200	28	—	No	3/5	—	I	7 14	—	IRDS (transient)
5	1205	30	Twin birth A	Moderate	1/30	—	I	7 25	164	IRDS (transient)
6	1000	26	Hydramnion	Severe	8/30	—	I	7 30	345	IRDS (transient)
7	920	33?	Twin birth A	Moderate	2/—	—	I	7 28	220	IRDS (transient)
8	1020	33?	Twin birth B	Moderate	2/—	—	I	7 35	480	Symptomless
9	1090	27	—	Moderate	2/10	—	—	7 25	—	Symptomless
10	1160	29	Twin birth B	No	2/5	—	I	7 21	—	Hypoglycemia
11	1040	32	Caes section	No	—/37	—	I	7 22	470	Hypoglycemia
12	960	31	Twin birth B	?	2/30	—	I	7 24	440	Symptomless
13	980	26	Breech delivery	Moderate	8/—	96	I	7 35	335	Apnea RLF
14	1120	27	—	No	14/—	21	I	7 24	340	Apnea BPD
15	1250	24?	—	No	6/25	78	I	7 19	215	Apnea IRDS (transient)
III *Slight or no RD*										
16	1150	32?	—	No	7/30	72	I	7 32	420	Apnea
17	1170	34	Maternal bleeding	No	—/40	—	—	7 32	435	Symptomless
18	1100	32	Breech delivery	No	—/50	—	I	7 22	420	Hypoglycemia
19	1050	28	—	No	11/30	—	I	7 32	290	Symptomless
20	1150	24	—	Moderate	2/45	—	I	7 25	290	Symptomless
21	1100	28	Ablatio plac	No	1/10	—	—	7 22	—	Symptomless
22	900	28	Twin birth B	No	19/5	—	I	7 34	490	Symptomless

RD The postmortem findings are discussed in detail in the second part of the study (26)

From Tables 2 and 3 it is clear that *severe RD is almost invariably fatal (only one survivor)* The majority of cases having *moderate RD* survived (14 of 21) whereas there were *more deaths (9 of 16) in the category of slight or no RD* This is caused by the occurrence of unexpected respiratory failure following a comparatively symptomless period in the fatal cases of intrauterine infection (4 cases) and intraventricular brain hemorrhage (2 cases)

As demonstrated in Table 4 the incidence of birth complications is markedly higher in category I (92) as compared with only 76 % and 63 in categories II and III respectively As could be expected there were more moderately or severely asphyxiated infants *in categories I and II* All cases of *severe RD* were treated on IPPR whereas 10 of 21 and 10 of 16 respectively required assisted ventilation *in categories II and III*

As also demonstrated in Tables 2 and 3 there was a good correlation between the degree of reticulo granular density in chest X rays and severe IRDS 9 of the 11 chest X rays revealed changes of grade II and III Of the surviving cases however only the case of severe IRDS also requiring IPPR showed the grade II changes In the cases of intrauterine infection the first chest X rays (obtained during the first 24 hours after birth) were considered normal *in all but two cases* and the patchy and lobar infiltrations indicating pneumonia were seen only during the later course *of the illness*

Table 3 *Deaths detailed clinical data grouped according to the three categories of respiratory distress*

(IRDS = idiopathic respiratory distress syndrome IVH = intraventricular brain hemorrhage INF = infection PH = massive pulmonary hemorrhage BPD = bronchopulmonary dysplasia)

Case no	B W (g)	Gest age	Complic factors at birth	Birth asphyxia	Age on admiss hr/min	Onset of IPPR (hr)	Chest X ray	Init art pH	Init art P_{O_2} mm Hg	Diagnosis
I *Severe RD*										
23	1100	34	Caes section	Severe	—/35		II	7 07	30	IRDS PH
24	870	28	Ablatio plac	Severe	1/—		II	6 99	—	IRDS
25	1000	26	Hydramnion	Severe	1/30	At birth	III	7 25	40	IRDS PH
26	1170	26	Early rupture of the membranes	Severe	—/30		—	6 55	33	INF IVH
27	1080	28	—	Severe	2/45		I	7 09	—	IVH
28	1090	26	Placenta previa	Moderate	5/—	6	III	7 06	59	IRDS
29	1210	24	Twin birth B	Moderate	4/—	4	III	7 17	78	IRDS
30	1230	29	Twin birth B	No	3/40	4	III	7 25	—	IRDS
31	880	25	Early rupture of the membranes	Moderate	5/50	6	II	6 85	21	INF
32	[illegible]	31	Twin birth A	No	2/—	14	III	7 06	80	IRDS BPD
33	1000	28	Early rupture of the membranes	No	—/40	1	I	6 86	26	IVH PH
II *Moderate RD*										
34	1200	28	Hydramnion	No	1/—	13	—	[illegible]	—	IRDS
35	850	27	Twin birth A	No	3/45	125	I	7 12	41	IVH pneumoth
36	1230	33	Breech delivery	Severe	2/45	3	—	7 31	470	IVH INF
37	1100	27	Early rupture of the membranes	No	7/10	25	II	7 07	66	INF IRDS
38	860	24	—	No	1/35	1	I	6 90	174	IRDS
39	1000	26	Caes section	Moderate	1/45	5	II	7 22	8	IRDS PH
40	990	27	Twin birth B	Moderate	3/18	111	I	7 21	260	IRDS IVH
III *Slight or no RD*										
41	1100	27	—	No	9/30	96	II	7 26	—	Apnea pneumoth
42	1100	[illegible]	—	No	1/43	96	II	7 28	360	IVH pneumoth
43	1050	6	Early rupture of the membranes	Moderate	3/—	6	I	7 04	200	IRDS INF
44	940	26	Caes section	No	—/20	15	I	7 31	250	Apnea INF (sec)
45	1110		Early rupture of the membranes	No	2/30	32	I	32	415	INF IVH
46	1000	25	Early rupture of the membranes	No	46/—	59	I	7 29	230	INF
47	1000	27	—	No	—/30	33	I	7 18	510	Apnea INF (sec)
48	1100		Early rupture of the membranes	Moderate	2/—	15	I	7 22	55	INF IRDS
49	890	[illegible]	Breech delivery	Moderate	1/25	28	—	7 22	—	IVH

In Table 5 the mean values of initial arterial pH P_{CO_2} and P_{O_2} (determined following a 10-minute period of breathing 90–100% oxygen) and the calculated R L shunt percentages *(16)* are given The most marked differences are seen in pH P_{O_2} and R L shunt values *in the expired cases of severe RD* pH values below *7 00* were found in 4 all P_{O_2} values were below 100 mm of mercury and *the R L shunt* percentage (mean) was 50* as compared with 28% in survivors Both pH and P_{O_2} were remarkably higher in the other two groups and there was also a striking difference between survivors and deaths As an interesting finding there were only 3 surviving infants with initial pH values below 7 20

Response to IPPR

In Fig 3 the response to mechanical ventilation as demonstrated by changes in arterial

Table 4 *Complicating factors at birth birth asphyxia and number of cases treated on IPPR in the 3 categories of respiratory distress (RD)*

		Severe RD	Moderate RD	Slight or no RD
Total number of infants		12	21	16
Complicating factors at birth		11 (92)	16 (76)	10 (63)
Birth asphyxia	(5 minute Apgar score)			
Severe	(0–3)	5	3	—
Moderate	(4–7)	4	7	3
No	(8–10)	3	11	13
Number of cases treated on IPPR		12	10	10

P_O P_{CO_2} and pH is graphically illustrated in four typical cases of apnea IRDS massive IVH and infection (pneumonia) It is clearly seen that the initial response to respirator treatment as far as P_O values are concerned as good in apnea and IVH whereas there is a moderate rise in the case of pneumonia but no rise in IRDS In contrast to the changes in P_O there are no corresponding differences between the four cases as demonstrated by rising pH and falling P_{CO} values The cases of apnea and IVH however seem to become hyperventilated as shown by low P_{CO} values (around 20 mm of Hg) and high pH values respectively

Results of treatment

Table 6 shows the mortality figures for each major cause of respiratory failure There were a total of 19 infants diagnosed as having IRDS of the 13 cases treated on IPPR only one survived Cases of intrauterine infection were invariably fatal as well as cases of massive IVH and of massive pulmonary hemorrhage Of the 6 cases treated on IPPR for apnea unrelated to IRDS or infection 4 survived and the two deaths followed complications of the treatment one died of pneumothorax and one of overwhelming infection There were 11 surviving cases, where no signs indicating pulmonary or extrapulmonary pathology could be demonstrated although some of these infants showed transient signs of *moderate RD* (5 cases) Arterial blood gases and pH were normal in these cases and the chest X rays were also considered normal

All survivors have been followed and the physical examination has been performed at the ages of 3 6 12 and 24 months The follow up study is still in progress There was one case of retrolental fibroplasia (case no 13 in Table 2) occurring in the first case of the present series

Table 6 *Results of treatment clinical/autopsy findings and outcome*

Clinical/Autopsy findings	Survivors	Deaths	Total
IRDS/HMD	7	12	19
Infection	—	8	8
Apnea	4	2	6
Massive IVH	—	4	4
Pulmonary hemorrhage	—	1	1
Symptomless	11	—	11

Table 5 *Initial values of pH P_{CO_2} and P after a 10 minute period of breathing 90–100 oxygen*

Data	Severe RD		Moderate RD		Slight or no RD	
	Surv	Deaths	Surv	Deaths	Surv	Deaths
Number of cases	1	11	14	7	7	9
Mean pH (range)	7 21	7 03 (6 55–7 25)	7 25 (7 14–7 35)	7 14 (6 90–7 22)	7 28 (7 2_–7 34)	7 4 (7 04–7 32)
Mean P_{CO} mm Hg (range)	62	76 (45–150)	53 (31–82)	70 (43–130)	44 (31–58)	62 (34–140)
Mean P_O mm Hg (range)	140	46 (24–80)	320 (164–480)	153 (8–4 0)	391 (?90–490)	297 (55–510)
Mean R L shunt (range)	28	50 (41–80)	20 (11–31)	27 (19–65)	17 (14–26)	4 (1 –56)

trated on IPPR who required respirator treatment for nearly four weeks. Only during the first of these weeks was it possible to obtain arterial samples. Thus the P_{O_2} values possibly responsible for the development of the retrolental fibroplasia are unknown. There was one case demonstrating the typical symptom-complex of the bronchopulmonary dysplasia (17) (case no. 14 (BPD) in Table 2). Case no. 6 showing signs of severe brain damage at the age of one year was found by pneumo-encephalography to have a brain malformation (single ventricle associated with cortical atrophy). The number of cases showing signs of slight or moderate brain injury is small as compared with the majority of cases where no neurologic abnormalities can be demonstrated.

DISCUSSION

It is well known that there is a better correlation between gestational age and perinatal mortality than between birth weight and perinatal mortality. As illustrated in Fig. 2, there was a much higher mortality rate in the low gestational age group (24 to 28 weeks) than in the small for date infants (29 to 34 weeks of gestation). Although the incidence of IRDS in different weight groups has been reported by several workers (2, 14, 15, 20) the correlation of the incidence of IRDS with gestational age and birth weight has only infrequently been given in infants weighing less than 1250 g at birth (9). In the present series there is a strikingly higher incidence of severe IRDS requiring IPPR in prematures with gestational ages below 29 weeks (10 cases of 23) than in infants with gestational ages over 29 weeks (3 of 23).

Etiologic factors

The causal relationship between complications during the delivery leading to intrauterine and postpartum hypoxia and the incidence of hyaline membrane disease is well documented (14, 16). As demonstrated in tabels 2 and 3, there were only a few cases of spontaneous uneventful deliveries in the present series. In all but one of the 12 cases of *severe RD* the delivery was complicated as compared with 6 uneventful deliveries out of 16 in cases of *slight or no RD*. Accordingly, of the 19 cases diagnosed as having IRDS only 3 were born without complicating factors at birth.

As shown by Gotzelin (11), there is a high incidence of intrauterine infection in cases of early rupture of the fetal membranes. Our findings support this view since in all but one of the 9 cases where there was a history of early rupture of the membranes extensive inflammatory changes were found at autopsy (26).

Prolonged birth asphyxia and hypoxia have been suggested by Harrison *et al.* (12) to be etiological factors leading to massive intracranial hemorrhages in cases of severe IRDS. In the present series, however, only one of the 4 cases of massive IVH occurring alone was severely asphyxiated at birth. Of the remaining 9 cases of IVH associated with pulmonary findings 6 suffered from moderate or severe birth asphyxia. On the other hand, many of the cases had apneic spells before the onset of IPPR and thus the brain hemorrhages may be also caused by hypoxia associated with apnea.

Respiratory symptoms

Irregular breathing including apneic spells during the first days of life is frequently seen in small otherwise symptomless surviving premature infants. Most of the diseases affecting prematures are also characterized by respiratory signs of varying severity. Thus owing to these findings the series was divided into three categories according to the severity of the respiratory symptoms during the first 12 to 24 hours after birth.

As shown in Table 4, all 12 infants in the category of *severe RD* suffered from respiratory failure requiring assisted ventilation; all but one died. As also discussed by Adamson and co-workers (1), these infants may be considered as "non salvageable" and thus could be left out of the respirator treatment. By contrast there were 10 cases treated on IPPR of 21 infants with *moderate RD* and also 10 cases

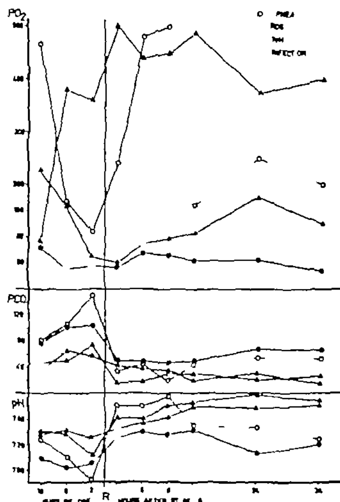


Fig 3 Response to mechanical ventilation changes in arterial Po (determined following a 10 minute period of breathing 90–100 oxygen) Pco_2 and pH following the onset of IPPR in illustrative cases of apnea IRDS IVH and infection (pneumonia)

of 16 with *slight or no RD* there were 7 and 9 deaths respectively There is an unexpected high incidence of respiratory failure in infants showing only *mild or transient respiratory* signs during the first 12 to 24 hours of life The high mortality rate may be caused by the fact, that in many cases of intrauterine infection (4 cases) and brain hemorrhage (2 cases) the period preceding a sudden attack of respiratory failure requiring IPPR was comparatively symptomless

Owing to the extreme immaturity of the infants in the present series, the main symptoms of severe IRDS were already indicative of respiratory failure In contrast to IRDS of the more mature infants there was seldom time enough for the *typical respiratory symptom* complex to develop, respirator treatment w started at the mean age of 7 hours On t other hand there were also cases showing t characteristic respiratory symptoms of IRI but where no pulmonary hyaline membra could be found at autopsy (26) Of the 6 m cases 4 showed only transient signs of IR like the cases reported by Downes *et al* (

Blood gases and pH

Initial arterial P_O levels in 100 amb oxygen concentration have been shown by B ton *et al* (4) to be a more reliable guide prognosis of IRDS than changes in pH a P_{CO} *On the other hand right to left shunt* is the dominant cause of hypoxemia (21 2 and thus the calculated R–L shunt percent *(17) is a useful additional indicator of the p* monary hypoperfusion (5) Wallgren and c workers have shown by direct measureme (25) that the extrapulmonary R–L shunt takes place mainly through the foramen ov rather than ductus arteriosus in *premature* fants with IRDS

The infants who died after *severe RD* in t *present series*, had a strikingly lower mean p (7 03) P_O (46 mm Hg) and accordingly a high R–L shunt percentage (50) as compared w deaths in the two other categories (pH 7 14 a 7 24 P_O 153 and 297 and R–L shunt * 27 a *24 respectively) There was only slight if a* difference between P_{CO} values The comparativ low P_{CO} in the expiring cases of severe IRI is caused by the fact that many of these infan (5 cases) were already on assisted ventilat when the first arterial samples for the *dete* mination of the blood gases and pH were take

Response to IPPR

The response to IPPR as demonstrated b changes in blood gases *and pH has be* shown to be a valuable prognostic paramet (1 6) This is also true for the extremely sma *prematures of the present series* but only a far as changes of the arterial P_O, are co cerned As illustrated in Fig 3 *there is a rap* rise in P_O values of the infants dying of bra

hemorrhage unrelated to IRDS These cases are sometimes almost impossible to distinguish from the cases suffering from severe apnea The cases of intrauterine infection show a moderate response to IPPR initially followed by a later downhill course of the illness In most cases of severe IRDS no rise in P_O values could be demonstrated

The only characteristic change in pH and P_{CO} values seems to be the tendency to low P_{CO} and accordingly high pH levels in cases of apnea and IVH In some cases it is striking enough to be of diagnostic value

Follow up study

A few follow up studies comprising premature infants weighing less than 1250 g at birth have been reported (8 13) Kamtero and co-workers (13) in a retrospective study of low birth weight infants found a high incidence of symptoms indicating brain injury (68) at the age of 6 to 7 years However the lack of detailed studies on blood gases and pH blood glucose levels and even bilirubin make it difficult to draw any conclusions about the possible etiological factors known to be responsible for major neurological damage On the other hand the intensive care of newborn infants including IPPR *might* result in increasing the incidence of brain damage by making it possible for the most severely affected infants to survive

The preliminary data obtained during the follow up study still in progress indicate however that there are only a few infants suffering from severe brain injury Most of the infants seen at the age of two years are indistinguishable from normal babies It is still far too early however to draw any conclusions about the survivors b cause as well known there may be many minor brain injuries which are only possible to find at a later age

SUMMARY

Clinical findings and results of treatment in a series of 49 premature infants with birth weights between 850 and 1250 g are presented The "intensive care included nursing in incubators with high environmental humidity maintaining skin temperature at 36 5 C umbilical catheterisation (both arterial and venous) intravenous glucose infusions correction of metabolic acidosis by rapid sodium bicarbonate infusions oxygen therapy and finally endotracheal intubation and mechanical ventilation

The series was divided into 3 categories according to the severity of respiratory symptoms There was only one survivor out of 12 cases in the category of *severe RD* whereas 14 of 21 with *moderate RD* and 9 of 16 with *slight or no RD* survived The overall survival rate was 45 The correlation between clinical findings arterial blood gases and pH chest X rays response to treatment and outcome in each of the 3 categories is discussed The preliminary data from our follow up studies indicate that there are only a few cases showing signs of permanent brain injury at the age of two years

REFERENCES

1 Adamson T M, Collins L M Hawker J M & Reynolds E O R Mechanical ventilation in newborn infants with respiratory failure *Lancet II* 227 1968
2 Avery M E & Oppenheimer E H Recent increase in mortality from hyaline membrane disease *J Pediat* 57 553 1960
3 Backstrom L & Kauppinen M A The intrauterine growth as estimated from birth weight data at 25 to 43 weeks of gestation for Finnish babies *Journal of the Finnish Medical Association* 23 1553 1968
4 Boston R W Geller F & Smith C A Arterial blood gas tensions and acid base balance in the management of the respiratory distress syndrome *J Pediat* 68 74 1966
5 Chu J Clements J A Cotton E Klaus M H Sweet A Y Thomas M A & Tooley W H The pulmonary hypoperfusion syndrome *Pediatrics* 35 733 1965
6 Cooke R Lunding M Lomholt, N F Ytting M Zachau-Christiansen B & Friis Hansen B Respiratory failure in the newborn *Acta Paediat Scand* 56 498 1967
7 Downes J J Arya S Morrow G & Boggs T R Transient respiratory distress syndrome in the newborn *Arch Dis Child* 42 659 1967
8 Drillien C M The incidence of mental and

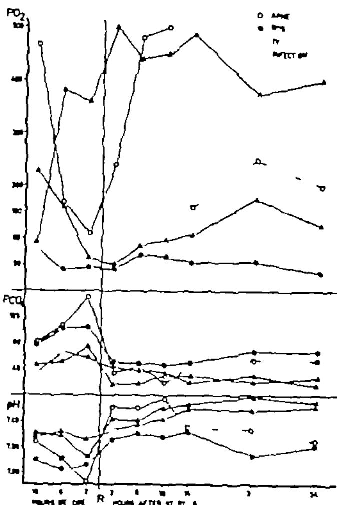


Fig 3 Response to mechanical ventilation changes in arterial Po (determined following a 10 minute period of breathing 90–100 oxygen) Pco and pH following the onset of IPPR in illustrative cases of ?pooa IRDS IVH and infection (pneumonia)

of 16 with *slight or no RD* there were 7 and 9 deaths respectively There is an unexpected high incidence of respiratory failure in infants showing only mild or transient respiratory signs during the first 12 to 24 hours of life The high mortality rate may be caused by the fact, that in many cases of intrauterine infection (4 cases) and brain hemorrhage (2 cases) the period preceding a sudden attack of respiratory failure requiring IPPR was comparatively symptomless

Owing to the extreme immaturity of the infants in the present series the main symptoms of severe IRDS were already indicative of respiratory failure In contrast to IRDS of the more mature infants, there was seldom time enough for the typical respiratory symptom complex to develop, respirator treatment was started at the mean age of 7 hours On the other hand there were also cases showing the characteristic respiratory symptoms of IRDS, but where no pulmonary hyaline membranes could be found at autopsy (26) Of the 6 mild cases 4 showed only transient signs of IRDS like the cases reported by Downes *et al* (7)

Blood gases and pH

Initial arterial P_O levels in 100 ambient oxygen concentration have been shown by Boston *et al* (4) to be a more reliable guide to prognosis of IRDS than changes in pH and P_{CO} On the other hand right to left shunting is the dominant cause of hypoxemia (21 22) and thus the calculated R–L shunt percentage (17) is a useful additional indicator of the pulmonary hypoperfusion (5) Wallgren and coworkers have shown by direct measurements (25) that the extrapulmonary R–L shunting takes place mainly through the foramen ovale rather than ductus arteriosus in premature infants with IRDS

The infants who died after *severe RD* in the present series had a strikingly lower mean pH (7 03) P_O (46 mm Hg) and accordingly a higher R–L shunt percentage (50) as compared with deaths in the two other categories (pH 7 14 and 7 24 P_O 153 and 297 and R–L shunt 27 and 24 respectively) There was only slight if any difference between P_{CO} values The comparatively low P_{CO} in the expiring cases of severe IRDS is caused by the fact that many of these infants (5 cases) were already on assisted ventilation when the first arterial samples for the determination of the blood gases and pH were taken

Response to IPPR

The response to IPPR as demonstrated by changes in blood gases and pH has been shown to be a valuable prognostic parameter (1 6) This is also true for the extremely small prematures of the present series but only as far as changes of the arterial P_O are concerned As illustrated in Fig 3 there is a rapid rise in P_O values of the infants dying of brain

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OSTEOBLASTS AND OSTEOCLASTS IN BONE MARROW ASPIRATED FROM CHILDREN WITH RICKETS

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[In]creased alkaline phosphatase activity in serum [(]SAP) is the most striking and consistent bio[c]hemical abnormality in rickets and forms one [o]f the early changes brought about by Vitamin [D] deficiency The rise in SAP tends to be [d]irectly related to the severity of the disease and is probably associated with the overpro[l]iferation of osteoblasts in the rachitic bones (7)

The present study attempts to demonstrate the osteoblastic proliferation in rickets by simple bone marrow aspiration thus providing an additional diagnostic aid It also tries to determine whether bone marrow findings are correlated to SAP levels on one hand and to other biochemical and roentgenological changes characteristic of the disease on the other

MATERIAL AND METHODS

A group of 21 children with rickets (Table 1) provided material for the present study Ten apparently healthy children at the age of 3 to 16 months without rickets formed the control group Calcium and inorganic phosphorus in serum were determined by the standard methods alkaline phosphatase was expressed in Bodansky units

In 17 cases bone marrow aspiration biopsy was performed from the superior anterior iliac crest using the usual procedure and in the 14 remaining cases from the tibial tuberosity The aspirated material was examined by phase contrast microscopy and stained with May Grunwald Giemsa stains In 17 cases of rickets smears were stained to demonstrate alkaline phosphatase activity by the Kaplow method (9) and in 4 cases staining for acid phosphatase activity was performed according to Rozenszajn (11)

Two smears from every case were carefully examined for the counting of osteoblasts and osteoclasts but since these cells are small in number and unevenly distributed on the smear counting in relation to other cells seems to provide inaccurate data Only the total number of osteoblasts and osteoclasts in the whole smear was therefore determined The cellularity of every smear was estimated by counting the total number of nucleated cells in three measured squares on a graticule in the eyepiece The mean number of nucleated cells was considered representative of the relative mean smear-cellularity

RESULTS

Osteoblasts are oval and sometimes elongated cells 25 to 50 μ in diameter with rather blurred outlines The cytoplasm in the May–Grunwald–Giemsa stains frequently assumes a light blue shade It contains few azurophilic granules and is occasionally fenestrated The nucleus lies excentrically and a lighter portion (the juxtanuclear "vacuole") which contains the Golgi bodies (1) is evident in the central part of the cytoplasm Phase contrast examination revealed that the cytoplasm contains many mitochondria (Fig 1) Osteoclasts are giant cells and their diameter often exceeds 100 μ Their outlines are distended and not very clear The cytoplasm is cloudy and finely granular in the marginal portion and in the May–Grunwald–Giemsa staining it appears from weakly basophilic to strongly acidophilic It contains numerous azurophilic granules of various sizes and many mitochondria These cells contain

physical handicaps in shool age children of very low birth weight *Pediatrics 27* 452 1961
9 Dunn P M The respiratory distress syndrome of the newborn immaturity versus prematurity *Arch Dis Child 40* 62 1965
10 Fisch R O Gravem H J & Engel R R Neurological status of survivors of neonatal respiratory distress syndrome *J Pediat 73* 3 1968
11 Gosselin O *Contribution à l'etude de l'invasion des organismes maternal et foetal par les microbes des voies genitales inférieure au cours du travail* Vaillant Carmanne S A Liege 1945
12 Harrison H Heese de V & Klein M Intracranial hemorrhage associated with hyaline membrane disease *Arch Dis Child 43* 116 1968
13 Kantero R L Frisk M & Holmstrom G Small prematures at 6-7 years of age IV *Ann Paediat Fenn 11* 175 1965
14 Keuth U *Das Membransyndrom der Fruh und Neugeborenen* Experimentelle Medizin Pathologie und Klinik Band *16* Springer Verlag Berlin
15 Miller H C Respiratory distress syndrome of newborn infants Diagnosis and incidence *J Pediat 61* 2 1962
16 — Respiratory distress syndrome of newborn infants Clinical study of pathogenesis *J Pediat 61* 9 1962
17 Nelson N M Prodhom L S Cherry R B Lipsitz P J & Smith C A Pulmonary function in the newborn infant the alveolar arterial oxygen gradient *J Appl Physiol 18* 534 1963
18 Northway W H Rosan R C & Porter D Y Pulmonary disease following respirator therapy of hyaline membrane disease Bronchopulmonary dysplasia *New Eng J Med 276* 7 1967
19 Siggaard Andersen O Engel K Jorgensen K & Astrup P A micro method for determination of pH carbon dioxide tension base excess and standard bicarbonate in capillary blood *Scand J Clin Lab Invest 12* 172 1960
20 Silverman W A & Silverman R H Incidence of hyaline membrane in premature infants *Lancet II* 588 1958
21 Strang L B The pulmonary circulation in the respiratory distress syndrome *Pediat Clin North Amer 13* 3 1966
22 Strang L B & MacLeish M H Ventilatory failure and right to left shunt in newborn infants with respiratory distress *Pediatrics 28* 17 1961
23 Thomas D V Fletcher G Sunshine P Schafer I A & Klaus M H Prolonged respirator use in pulmonary insufficiency of newborn *JAMA 193* 3 1965
24 Tunstall M E Cater J I Thomson J S & Mitchell R G Ventilating the lungs of newborn infants for prolonged periods *Arch Dis Child 43* 486 1968
25 Wallgren G Hanson J S Tabakin B S Raiha N & Vapaavuori E K Quantitative studies of the human neonatal circulation V Hemodynamic findings in premature infants with and without respiratory distress *Acta Paediat Scand Suppl 179* 1967
26 Vapaavuori E K & Krohn K Intensive care of small premature infants II Postmortem findings To be published

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Fig 2 Osteoclast phase contrast microscopy 1,250

ber of osteoblasts in the same smear ($r = -0.09$ in the minimal values and $r = +0.18$ in the maximal values) Nor did bone marrow puncture site (tibia or ileum) affect the number of osteoblasts and osteoclasts in the aspirated material

The ratio between the number of osteoblasts in bone marrow of the rachitic cases and the level of SAP calcium and phosphorus in their serum was calculated Significant correlation was found for SAP ($r = +0.61$ $p < 0.01$) (Fig 5) Results for calcium ($r = -0.28$) and phosphorus ($r = +0.07$) however are not significant

Table 2 *Osteoblasts osteoclasts and relative mean cellularity in bone marrow of the rickets group (I ileum T – tibia)*

No	Site of BM aspiration	Nucleated cells per 0 666² mm²	Osteoblasts per smear	Osteoclasts per smear
1	I	386–597	30–55	2 3
2	I	478 544	5– 7	0–1
3	I	790–874	29–30	2 5
4	I	790–850	17 15	1 2
5	I	277 370	15–20	0–1
6	I	833–836	33–79	10–11
7	T	600–632	19–30	0–2
8	I	166–186	11 11	0–2
9	T	730–880	15 20	0–3
10	T	379–360	42–44	0–2
11	I	140–168	35 45	1 1
1	I	17 190	19–20	1 1
13	T	181 200	15–23	0–2
14	T	210–217	23–32	–5
15	T	100–112	18 32	1 2
16	I	60– 66	54–55	5–8
17	T	460–574	19–26	0–0
18	I	2.8– 48	4– 7	0–0
19	T	45– 50	45–49	0–1
0	T	878–824	30–74	0–2
1	I	124–139	8–10	0–0

Table 1 *Clinical and laboratory Data obtained from 21 Children with Ricket s*
Ct = craniotabes Ros = rosary WW = wrist widening)

o	Sex	Age in months	Diagnosis	Clinical signs of Rickets Ct	Ros	WW	X Ray wrist	Ca mg/100 ml	P mg/100 ml	Alkal P Ac (B U)
	M	4 5	Pneumonia Anemia Iron def	+	+	±	±	10	6 2	27 5
	F	8	Pneumonia Anemia Iron def	+	+	+	+ +	6 0	3 7	40 2
	M	10	Pneumonia	−	−	+	+	8 2	3 9	22 0
	M	7	Cong cataract	+ +	−	−	±	11 5	4 4	70 0
	F	4 5	Pneumonia Gastroenteritis	−	+	+	+	10 2	6 3	27 3
	F	30	Vit D resist rickets	−	+ +	+ +	+ + +	5 7	5 8	83 5
	F	4	Pneumonia Gastroenteritis	+	+	+	±	6 5	4 2	70 0
	M	7	Mental retardation	−	+	±	±	6 7	4 4	21 7
	F	3	Gastroenteritis	+	±	±	+	10 0	3 3	23 2
	M	10	Pneumonia Anemia Iron def	+	+	+	±	10 0	5 2	37 4
	F	11	Familial dysautonomia	+	−	−	+	6 7	4 1	24 8
	M	5 5 yrs	Vit D resist rickets	−	+	+	+ +	10 2	2 1	26 8
	F	7	Pneumonia	+		+	+	10 6	3 0	34 6
	F	3	Cerebral palsy	+	+	+	±	8 1	5 5	16 5
	F	12	Anemia Iron def	+ +	+	+	+ +	9 2	2 8	65 5
	F	16	Anemia Iron def	±	+ +		+	7 5	2 3	46 6
	M	7	Pneumonia	+	±	+	±	8 8	5 7	16 7
	F	8	Pneumonia	+	±	±	+	10 0	3 8	14 7
	F	8	Pneumonia	+	±	±	+	10 2	4 4	28 7
	M	4	Pneumonia Tetany	+		+	+	5 4	5 1	40 0
	M	4	Pneumonia		−	+	+	5 2	6 2	16 0



1 Osteoblast phase contrast microscopy × 1 250

several nuclei which are loosely scattered over the whole cell and do not touch one another (Fig 2)

Results of osteoblast and osteoclast countings are summarized in Tables 2 and 3 *Osteoblasts* were pres nt in every case of rickets at an aver age number of 27 19 and ranged from 4 to 79 cells per smear They were however detected in only 5 cases of the control group and then always less than 4 cells per smear The increase in number of osteoblasts in rachitic bone mar row is statistically significant (Fig 3) *Osteo clasts* were present in 19 cases of rickets and ranged from 1 to 11 cells per smear No osteo clasts were found in the marrow of control subjects

No correlation could be established between the relative cellularity of smears (expressed by the mean number of nucleated cells per 0 666 mm on 3 regions of the smear) and the num



Fig 2 Osteoclast phase contrast microscopy 1,250

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Table 3 *Osteoblasts osteoclasts and relative mean cellularity in bone marrow of the control group (I = Ileum T = Tibia)*

No	Site of BM aspiration	Nucleated cells per 0 666² mm³	Osteoblasts per smear	Osteoclasts per smear
1	I	200–256	0–0	0
2	T	265–366	0–0	0
3	I	368–441	0–0	0
4	T	720 732	0–2	0
5	T	83–164	0–1	0
6	T	622 872	1–1	0
7	I	52– 61	0–1	0
8	I	172–180	1–1	0
9	T	57– 80	0–0	0
10	I	400–440	3–4	0

In the 7 cases which were radiologically evaluated as mild rickets the mean number of osteoblasts in the marrow was 24 93 per smear whereas in the 14 cases of florid radiological rickets the average was 28 43 per smear The *t* test showed that no significant difference exists between these two means

Osteoblasts consistently showed a positive cytochemical alkaline phosphatase activity in all rachitic marrows The reaction ranged from few cells with small faintly or moderately stained granules to many cells packed with brilliant brown–black large sized granules (Fig 5) The reaction in osteoclasts was negative An intense acid phosphatase activity appearing as large red–violet granules with marked diffusion towards the periphery was demonstrated in the osteoclasts (Fig 6) A weak reaction was also found scattered in the osteoblasts

DISCUSSION

Osteoblasts and osteoclasts are only rarely found in the bone marrow of healthy infants and adults Hanick & Libansky (6) aspirated samples of bone marrows from healthy individuals and detected osteoblasts in only 10% and osteoclasts in only 0 7% of the cases This also confirms the findings of the present study which indicate a highly significant increase in the number of such cells in bone marrow of rachitic children

Two mechanisms may provide explanation for the above observations One is related to the fact that the essential lesion of rickets is the accumulation of uncalcified osteoid which is formed by osteoblasts on the surface of the trabeculae in the shaft of the long bones

In reaction to the muscle stress imposed on the rachitic bones, excessively wide borders of

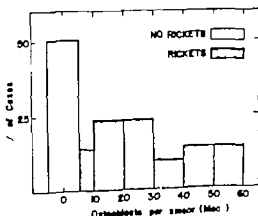


Fig 3 Distribution of osteoblasts per smear of bone marrow in the two groups of children

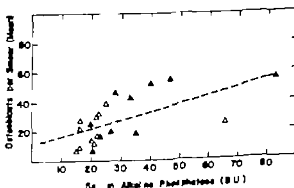


Fig 4 Correlation between SAP level and number of osteoblasts per smear of bone marrow in 21 cases of Rickets

osteoid with plenty of osteoblasts are formed around the trabeculae (7) The abundance of osteoblasts on the inner surface of the bone cortex is probably reflected in the aspirated bone marrow material

Another mechanism which might contribute to the osteoblastic and osteoclastic proliferation is associated with an increased production of parathyroid hormone stimulated by a reduction in calcium absorption from the intestines (5) There is a rapid proliferation of osteoclasts followed by a compensatory over proliferation of osteoblasts In extremely severe rachitogenic hyperparathyroidism can be recognized clinically In such cases (6) characteristic roentgenographic and histologic changes are present in the bone accompanied by massive infiltration of osteoblasts and osteoclasts into the bone marrow

A literature review revealed only few reports of diseases which are accompanied by high concentration of osteoblasts and osteoclasts in the



Fig 5 Osteoblast showing a marked positive alkaline phosphatase activity Kaplow method 1,250



Fig 6 Osteoclast with prominent positive acid phosphatase activity Rozenszajn method 1 250

bon marrow All the cases reported were characterized by bone destruction and by compensatory bone repair Rubinstein *et al* (12) reported 8 cases of osteitis deformans (Paget s disease) in which osteoblasts and osteoclasts were found in abundance in he bone marrow aspirated from affected bones only This *is an* obvious finding in such localized condition whereas rickets is a generalized disease The authors also report the presence of few osteoblasts in the marrow of a patient having bone metastases from a breast cancer as well as in other patients suffering from myeloid leukemia and Thalassemia major

Hanick & Libansky (6) postulated that numerous osteoblasts and few osteoclasts could be demonstrated in marrow of patients with osteoporosis metastazing carcinoma and Paget s disease while few such cells were ob-

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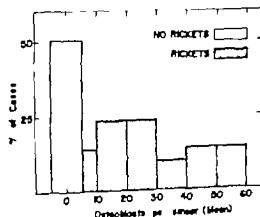


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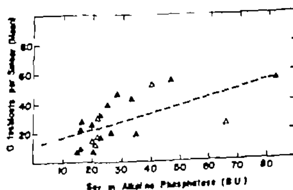


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served in case of Hodgkin s disease acute leukemia polycythemia vera and myelofibrosis They also noted this finding in four cases of rickets without stating the number of cells found Two other works which were published in European literature (3, 4) report an occasional finding of osteoblasts and osteoclasts in the marrow of few rachitic infants

Histologic bone sections showed that osteoblasts evince strongly positive alkaline phosphatase activity (10) while osteoclasts show intense acid phosphatase activity (2) This was further confirmed by the present study due to the increase in numbers of such cells in the rachitic marrow In contrast to previous observations (2) osteoblasts also showed faint positive acid phosphatase activity

Results obtained in the course of the present study *indicate that the increase in SAP level in* rickets is due to osteoblastic proliferation which is reflected in the aspirated bone marrow and in cytochemical staining This is in accordance with the demonstration accomplished by the starch gel electrophoresis technique showing that a separate isoenzyme of SAP is derived from the osteoblasts and excreted in increased amounts in rickets cases (13) It may therefore be concluded that in cases of unexplained high SAP level *an examination of aspirated bone* marrow for presence of osteoblasts and osteoclasts which is conducted parallel to the SAP electrophoretic technique may be of value in providing confirmatory evidence for the osteoblastic origin of high SAP thus pointing to the diagnosis of metabolic bone disorder

SUMMARY

Examinations of bone marrow samples aspirated from 21 infants with symptoms of rickets showed a significant increase in the number of osteoblasts and osteoclasts as compared with control material

The osteoblasts showed marked positive alkaline phosphatase staining reaction while the osteoclasts—and osteoblasts to a lesser extent—showed positive staining reaction of acid phosphatase

A significant correlation could be established between number of osteoblasts in bone marrow smears and level of serum alkaline phosphatase

Previous reports of similar marrow findings in diseases characterized by bone destruction and compensatory bone repair are reviewed The mechanisms and the possible significance of such findings are evaluated

REFERENCES

1 Arnott H J & Pautard F G E Osteoblast function and fine structure *Israel J Med Sci 3* 657 1967
2 *Burstone M S Histochemical demonstration of* acid phosphatase activity in osteoclasts *J Histochem Cytochem 7* 39 1959
3 Cazal P & Bertrano L Les cellules de la zone osteoide du cartilage de conjugaison dans le myelogramme tibial de l enfant *Rev Hemat 1* 293 1946
4 Eser M Osteoblasten und Osteoclasten in Knochenmarkausstrichen *Helvet Med Acta 12* *265 1945*
5 Fanconi G Physiology and pathology of calcium and phosphate metabolism *Advances Pediat 12* 307 1962
6 Hanick Z & Libansky J The cytomorphology of osteoblasts and osteoclasts *Acta Haemat 21* 366 1959
7 Harrison M E Rickets In *Brenneman s Practice of Pediatrics* W F Prior Co Inc 1963 vol 1 chapt 36
8 Illig R Uehlinger E & Prader A Sekundarer Hyperparathyroidismus bei Vitamin D-Mangel Rachitis *Helvet Pediat Acta 14* 566 1959
9 *Kaplow L S* A biochemical procedure for localizing and evaluating leukocyte alkaline phosphatase activity in smears of blood and marrow *Blood 10* 1023 1955
10 *Prichard J J A cytological and histochemical* study of bone and cartilage formation in the rat *J Anat 86* 259 1952
11 Rozenszajn L Marshak G & Efrati P Acid phosphatase activity in normal human blood and bone marrow cells as demonstrated by the AZO dye method *Acta Hemat 30* 310 1963
12 Rubinstein M A Smelin A & Freedman A L Osteoblasts and osteoclasts in bone marrow aspiration *Arch Int Med 92* 684 1953
13 Taswell H F & Jeffers D M Isoenzymes of alkaline phosphatase in hepatobiliary and skeletal diseases *Amer J Clin Path 40* 349 1963

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FIBRINOLYSIS IN HUMAN FOETUSES

H EKELUND ULLA HEDNER and B ÅSTEDT

From the Coagulation Laboratory of the Department of Paediatrics and the Department of Obstetrics and Gynaecology General Hospital Malmö Sweden

Our knowledge of *fibrinolysis in the human* foetus is incomplete A few investigations are however on record Thus Zilliacus *et al* (19) assessed the fibrinolytic activity by measuring the whole blood clot lysis time in 16 foetuses with crown heel lengths between 9 4 and 25 5 cm They recorded short times ranging from 20 to 120 min Some of the components of the fibrinolytic system have been studied by Ambrus *et al* (1) and Gitlin & Biasucci (8) As to plasminogen the former were not able to demonstrate any measurable amounts in 8 foetuses in the 4th to 8th month of gestation while the latter who used an immunological technique obtained values corresponding to about 20 per cent of the adult level in 13 foetuses in the 10th to 26th week of gestation As to the inhibitors of fibrinolysis Ambrus *et al* found the antiplasmin values to reach adult level already by the 4th month of gestation Gitlin & Biasucci found the α_2-macroglobulin to range from 17 to 42 per cent of the adult value and the α_1 antitrypsin from 56 to 92 per cent

Vahlquist *et al* (18) studied the fibrinogen in 8 foetuses with the gestational ages of 15 to 18 weeks and crown-heel lengths between 16 and 28 cm They found numerically lower values than in full term newborns (median values 0 18 g/100 ml and 0 39 g/100 ml respectively) but regarded the series too small to warrant statistical analysis

Judging from this brief survey then a more *detailed investigation* of the foetal development of the fibrinolytic system would be of interest and might also serve as a basis for further study of the subject in prematures

MATERIAL AND METHODS

The material consisted of 74 foetuses (including one pair of twins) obtained by induced abortion The mothers of 73 were healthy and their pregnancies had been terminated on socio-medical grounds One pregnancy was terminated because of suspected maternal rubella Since the foetus appeared normal it was included in the material Neither were any of the other foetuses malformed Routine pathological examination of the placentae revealed nothing remarkable

All the foetuses were delivered by abdominal hysterotomy under general anaesthesia with O_2+N_2O and fluothane Immediately after extraction of the foetus and the placenta the umbilical cord was divided and under sterile precautions a plastic catheter (French No 3 1/2) was introduced into one of the umbilical arteries Blood was drawn with a disposable plastic syringe This technique was successful in all but the smallest foetuses where the samples were obtained by heart puncture

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The blood was collected in siliconized glass tubes and in disposable plastic tubes Citrated plasma and serum were prepared in the way described previously (12 15) The following determinations were made with the methods described earlier (4) except for the antiplasmin assay

1 *Fibrinolytic activity* fibrin plate method (3)
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Histologic bone sections showed that osteoblasts evince strongly positive alkaline phosphatase activity (10) while osteoclasts show intense acid phosphatase activity (2) This was further confirmed by the present study due to the increase in numbers of such cells in the rachitic marrow In contrast to previous observations (2) osteoblasts also showed faint positive acid phosphatase activity

Results obtained in the course of the present study indicate that the increase in SAP level in rickets is due to osteoblastic proliferation which is reflected in the aspirated bone marrow and in cytochemical staining This is in accordance with the demonstration accomplished by the starch gel electrophoresis technique showing that a separate isoenzyme of SAP is derived from the osteoblasts and excreted in increased amounts in rickets cases (13) It may therefore be concluded that in cases of unexplained high SAP level, an examination of aspirated bone marrow for presence of osteoblasts and osteoclasts which is conducted parallel to the SAP electrophoretic technique may be of value in providing confirmatory evidence for the osteoblastic origin of high SAP thus pointing to the diagnosis of metabolic bone disorder

SUMMARY

Examinations of bone marrow samples aspirated from 21 infants with symptoms of rickets showed a significant increase in the number of osteoblasts and osteoclasts as compared with control material

The osteoblasts showed marked positive alkaline phosphatase staining reaction while the osteoclasts—and osteoblasts to a lesser extent—showed positive staining reaction of acid phosphatase

A significant correlation could be established between number of osteoblasts in bone marrow smears and level of serum alkaline phosphatase

Previous reports of similar marrow findings in diseases characterized by bone destruction and compensatory bone repair are reviewed The mechanisms and the possible significance of such findings are evaluated

REFERENCES

1 Arnott H J & Pautard F G E Osteoblastic function and fine structure *Israel J Med Sci 3* 657 1967
2 Burstone M S Histochemical demonstration of acid phosphatase activity in osteoclasts *J Histochem Cytochem 7* 39 1959
3 Caril P & Bertrano L Les cellules de la zone osteoide du cartilage de conjugaison dans le myelogramme tibial de l enfant *Rev Hemat 1* 293 1946
4 Esser M Osteoblasten und Osteoclasten in Knochenmarkausstrichen *Helvet Med Acta 12* 265 1945
5 Fanconi G Physiology and pathology of calcium and phosphate metabolism *Advances Pediat 12* 307 1962
6 Hanick Z & Libansky J The cytomorphology of osteoblasts and osteoclasts *Acta Haemat 21* 366 1959
7 Harrison M E Rickets In *Brenneman s Practice of Pediatrics* W F Prior Co Inc 1963 vol 1 chapt 36
8 Illig R Uehlinger E & Prader A Serum *darer Hyperparathyroidismus bei Vitamin D Mangel Rachitis* *Helvet Pediat Acta 14* 566 1959
9 Kaplow L S *A biochemical procedure for localizing and evaluating leukocyte alkaline phosphatase activity in smears of blood and marrow* *Blood 10* 1023 1955
10 Prichard J J A cytological and histochemical study of bone and cartilage formation in the rat *J Anat 86* 259 1952
11 Rozenszajn L Marshak G & Efrati P Acid phosphatase activity in normal human blood and bone marrow cells as demonstrated by the AZO-dye method *Acta Hemat 30* 310 1963
12 Rubinstein M A Smelin A & Freedman A L Osteoblasts and osteoclasts in bone marrow aspiration *Arch Int Med 92* 684 1953
13 Truswell H F & Jeffers D M Isoenzymes of alkaline phosphatase in hepatobiliary and skeletal diseases *Amer J Clin Path 40* 349 1963

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Table 1 *Distribution of the material*

Reported pregnancy week	Crown-heel length	No of foetuses	No of determinations of: Plasminogen	Antiplasmin	α_2 macroglobulin	Inhib of urokinase activ of plasminogen	Fibrinol activity Fibrin plates	Euglob clot lysis time	Fibrinolytic split products: serum	serum EACA
12–13	10	1	1	—	—	—	—	—	—	1
13–14	11	1	1	—	—	—	—	—	—	—
15	13	1	—	1	1	—	—	—	1	—
17 15 15	14	3	3	—	1	—	—	—	2	3
17 16	15	2	1	—	1	—	—	—	1	1
16–17 16 15	16	3	3	—	1	—	1	1	1	3
19 17 17 16 16	17	5	4	—	1	1	—	—	2	4
19 18 18 18 17 16 13	18	7	6	1	2	—	1	—	3	6
19 19 19 18 16	19	5	5	3	3	—	—	—	5	5
19 20 19 18 18 18 18?	20	6	6	5	5	—	1	—	6	6
19 19 19 19	21	4	2	2	2	—	1	1	2	2
20–21 19–20 19–20	22	3	2	2	2	2	—	—	[illegible]	2
20 20 20 19 19 18–19	23	6	6	5	5	3	5	2	6	6
21 20–21 20 20 20 20										
20 19 20 19–20 19	24	10	10	3	7	3	2	1	7	8
22 22 20 18–19	25	4	3	4	4	2	3	2	4	3
20–24 20 20 19?	26	4	3	1	1	3	1	—	2	3
23 21?	27	2	1	1	1	1	—	—	1	1
23 20?	28	2	2	2	2	1	1	—	2	2
24	29	1	1	—	1	—	1	1	1	1
24 20	30	2	1	2	2	2	—	—	2	1
23–24	31	1	1	1	1	1	—	—	1	1
18	32	1	1	1	1	1	1	—	1	1
Total number		74	63	34	44	20	18	8	52	60

3 *Fibrinolytic split products* (FSP) in serum and in serum from blood collected in a tube with an inhibitor of fibrinolysis (EACA)— serum EACA — immunochemical method (11) Values above 50 mg/100 ml were noted only as > 50 mg/100 ml since further dilution of the serum does not permit reliable quantitation Since the difference between the amount of FSP in serum and that in serum EACA reflects the plasminogen activator activity we used the difference between pairs of such samples as a measure of the fibrinolytic activity

4 *Plasminogen* immunochemical method (4 6)

5 *α-macroglobulin* esterolytic method (5)

6 *Antiplasmin activity* (progressive antiplasmin") A fibrin plate method requiring only 0 1 ml of serum was used instead of the caseinolytic method (4) A purified plasmin preparation (*Grade A freeze dried* 15 Sgouris CU/mg protein AB Kabi Stockholm) was used in a dilution of 12 5 Sgouris CU/ml 0 4 ml of this solution was added to the serum sample After incubation for 10 min 10 μl of each sample was applied to heated fibrin plates prepared according to Nilsson & Olow (14) The size of the lysed area after 18–20 hours in 37 C was taken as a measure of the remaining plasmin activity Pooled sera from 20 apparently healthy persons were used as control material A standard curve was obtained by plotting the results obtained simultaneously with various concentrations of plasmin (dil 1/1 = 100 per cent 1/2 = 50 per cent 1/4 = 25 per cent) incubated with 0 9 per cent NaCl The residual amount of plasmin was read against this curve and the result is expressed as a percentage of that in the pooled normal serum Normal range 60–140 per cent

7 *Inhibitors of the urokinase activation of plasminogen* clot method (16)

8 *Fibrinogen* spectrophotometric method (13) According to this method the fibrinogen values are calculated for a haematocrit of 40 per cent In this material the haematocrit was estimated in only 7 cases It ranged from 28 to 47 per cent The median *as well as the mean was 37 per cent* We therefore used the same correction factor

9 *Total serum protein* biuret method

10 The *haematocrit* was determined with a micro capillary centrifuge (Cellokrit AB Lars Ljungberg & Co Stockholm)

Figs 1–4 give normal values for newborns (4) and adults

Fibrinogen	Haematocrit	Total serum protein
—	—	—
—	—	—
—	—	—
—	—	—
—	—	—
—		—
—	1	—
—	—	1
2	1	3
—	—	2
—	—	—
—	1	—
[illegible]	2	3
4	1	1
1	—	4
1	1	1
1	—	1
1	—	1
1	—	—
[illegible]	—	—
—	—	—
1	—	—
	7	17

Statistical method

A rank method was used for measuring the correlations of plasminogen, the inhibitors of fibrinolysis, fibrinogen and total serum protein with the crown-heel lengths of the foetuses. This method was chosen in order to avoid undue influence of very low or high absolute values.

RESULTS

Table 1 gives the reported week of pregnancy at abortion ("menstruation age"), crown-heel (CH) lengths of the foetuses and number of analyses in each "length" group. Since it is known from experience that information about the duration of pregnancy week is unreliable especially when given by mothers with large foetuses, the results of all analyses were correlated with the CH lengths.

Fibrinolytic activity on fibrin plates (Fig. 1) was measured in 18 cases. The area lysed by plasma on unheated plates ranged between 38 and 486 mm² and by the resuspended euglobulin precipitate between 42 and 608 mm². In four subjects the activity of plasma exceeded that of the euglobulin fraction. There was also a small plasmin activity on heated plates (0–44 mm² for plasma, 0–48 mm² for resuspended euglobulin precipitate).

The euglobulin clot lysis time (Fig. 1) was measured in 8 of the 18 cases. It ranged from 6 to 21 minutes.

Fibrinolytic split products (FSP) were studied in 68 foetuses. The determinations were made in serum in 52 and in "serum EACA" in 60 cases. Pairs of samples were obtained from 44 foetuses. The results are given in Figs. 1 and 2.

FSP in serum were found in all cases except one. The amounts ranged between 2 and > 50 mg/100 ml.

Serum EACA contained at most traces of FSP in most cases. 21 samples contained between 1 and 5 mg/100 ml. 6 and 12 mg/100 ml were found in two foetuses where the corresponding values in serum were 50 mg/100 ml and 15 mg/100 ml respectively. It can be questioned whether moderate amounts of FSP found in serum EACA reflect the conditions *in vivo*. Sampling difficulties sometimes delayed transfer of the blood from the catheter and syringe to the tube with EACA. During this interval fibrinolytic activity might have produced FSP before being inhibited by EACA.

Plasminogen (Fig. 3), determined in 63 cases, ranged from 7.5 to 50 per cent with a median of 20 per cent. The values did not vary significantly with CH length ($r = +0.06$, $p > 0.10$).

Inhibitors of fibrinolysis: *α_2-macroglobulin* (Fig. 3) as assayed in 44 cases ranged from 5 to 63 per cent with a median value of 36 per cent. There was a significant increase of the values with the CH length ($r = +0.35$, $0.01 < p < 0.05$). *Antiplasmin* (Fig. 3) was determined in 34 cases. The range was wide, 29–1000 per cent, owing to the occurrence of two low (29 and 33 per cent) and two extremely high values (580 and 1000 per cent), the latter two in

Fig 1 Fibrinolytic activity on fibrin plates in 18 foetuses Comparison with euglobulin clot lysis time and FSP in some of the cases *O* = no measurable amount of activity *T* = traces of FSP – = determination not done

the twins The median was 100 per cent The antiplasmin did not vary significantly with CH length ($r = +0\ 09$ $p > 0\ 10$) *Inhibitors of urokinase activation of plasminogen* (Fig 3) were estimated in only 20 cases because the method used requires 0 5 ml of serum The range found was rather wide (93–768 per cent) with a median of 232 per cent The values did not vary significantly with CH length ($r = -0\ 31$ $p > 0\ 10$)

Fibrinogen (Fig 4) as assayed in 18 cases ranged from 0 03 to 0 31 g/100 ml with a

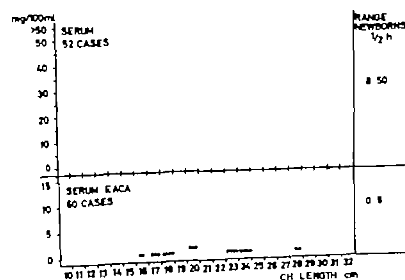


Fig 2 Fibrinolytic split products (mg/100 ml) in serum from 52 foetuses and in serum EACA from 60 foetuses

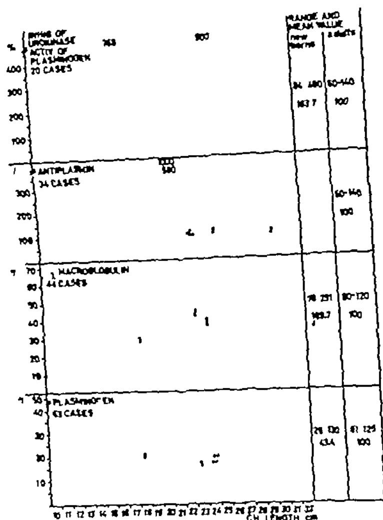


Fig. 3. Determinations of plasminogen and the inhibitors of fibrinolysis.

median value of 0.09 g/100 ml. The values did not increase with the size of the foetus ($r = +0.36$, $p > 0.10$).

Total serum protein (Fig. 5) in 17 cases studied ranged from 3.0 to 4.5 g/100 ml with a median value of 3.4 g/100 ml. It did not vary with CH length ($r = +0.17$, $p > 0.10$).

Haematocrit (Fig. 5) was estimated in only 7 cases. The range was 28–47 per cent with a median value of 37 per cent.

DISCUSSION

We demonstrated the occurrence of plasminogen, plasminogen *activator activity*, *inhibitors* of fibrinolysis and fibrinogen as well as a considerable fibrinolytic capacity in human foetuses from a CH length of 10 cm (corresponding to about 13 weeks of gestation).

We calculated the fibrinolytic activity from lysis on fibrin plates, from the euglobulin clot lysis time, and from FSP in serum and serum EACA.

As measured on unheated fibrin plates the fibrinolytic activity was considerable. We also found large amounts of FSP in serum, but usually at most small amounts in serum EACA. Taken together, these *findings* argue clearly for a high plasminogen activator activity, which appeared to be of the same level in all the

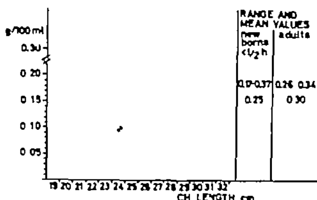


Fig 4 Fibrinogen values in 18 foetuses

foetuses studied The euglobulin clot lysis time was studied in only a few cases It was short and in agreement with the other findings The fibrinolytic activity did not increase with the size of the foetus In most cases the activity reached the same level as that during the first few hours of life in normal newborns (4)

The plasminogen levels measured by an immunochemical method showed a median value of 20 per cent of the adult level without any significant increase during the gestational period studied Our results agree well with those reported by Gitlin & Biasucci (8) who used radial immunodiffusion for quantitation of the proteins Ambrus *et al* (1 2) were not able to demonstrate any plasminogen at all until shortly before term apparently because the levels were below the sensitivity of the methods used (2)

The content of inhibitors of plasminogen activation was the same as in newborns (4) and thus larger than in adults

The level of α macroglobulin was lower than in newborns (4) It was the only factor in the fibrinolytic system that significantly increased with the size of the foetus This variation of α macroglobulin with postnatal growth is well known (7) but its physiological significance is obscure Our values agree with those of Gitlin & Biasucci (8)

Progressive antiplasmin was of adult level and did not vary with the size of the foetus This result agrees with that of Ambrus *et al* (1) who found antiplasmin at adult levels in the fourth gestational month Since this antiplasmin activity is located in the α_1 antitrypsin fraction our results may also be compared with the immunologically quantitated amounts of the protein reported by Gitlin & Biasucci (8) For the corresponding gestational period several values reached 80 to 92 per cent of the adult level Our results agree fairly well with their determinations

To conclude except for α macroglobulin there was thus an adequate inhibitor level probably as in the newborns, to counteract the high plasminogen activator activity

As to fibrinogen we could not find any data concerning human foetuses since the report of Vahlquist *et al* (18) in 1953 They studied 8 foetuses (CH lengths 16–28 cm) and found with a spectrophotometric method levels between 0 05–0 29 g/100 ml with a median value of 0 18 g/100 ml In our 18 cases we found a range between 0 03 and 0 31 g/100 ml with a median value of 0 09 g/100 ml There was no significant variation with the size of the foetus Gitlin & Biasucci (8) did not study plasma fibrinogen but demonstrated synthesis of fibrinogen in liver tissue of embryos in the fifth week of gestation

In conclusion foetal blood contains plasminogen and a definite plasminogen activator activity which can together produce a remarkable fibrinolytic activity These findings do not support the hypothesis of Ambrus *et al* (2) on the pathogenesis of the hyaline membrane syndrome According to them the premature infants have virtually no circulating plasminogen and the mechanism for removal of fibrin deposits in alveoli may therefore not function In our opinion there is no such deficiency in these in



Fig 5 Total serum protein in 17 foetuses and haematocrit in 7 foetuses

fants since the fibrinolytic system is already developed As a matter of fact Ludwig (10) has produced evidence for enhanced fibrinolysis in hypoxic newborns

The fibrinolytic mechanism has recently been discussed by Sharp (17) He pointed out the physiologic significance of fibrinolysis in preventing fibrin formation in the circulation

Since enzyme systems essential to life are active early in the foetus (9 16) one might tentatively assume that the fibrinolytic system develops very early in order to maintain patency of the foetomaternal circulation and remove fibrin deposits from placental villi Our investigation has demonstrated a developed fibrinolytic system already in early foetal life possibly to serve this important function

SUMMARY

The fibrinolytic system was studied in blood from 74 human foetuses in the 12th to 24th gestational week and with crown heel lengths between 10 and 32 cm

Plasminogen could be demonstrated in all foetuses in an amount equal to about 20 per cent of that in adults There was also a definite plasminogen activator activity and a remarkable fibrinolytic capacity The α₂-macroglobulin content was about 30 per cent of that in adults while progressive antiplasmin was at and the inhibitors of plasminogen activation above adult level

The fibrinolytic system develops early in intrauterine life Like other enzyme systems essential to life it must serve some physiologic purpose presumably to keep the foetomaternal circulation patent

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REFERENCES

1 Ambrus C M Weintraub D H Niswander K R & Ambrus J L Studies on hyaline membrane disease II The ontogeny of the fibrinolysin system *Pediatrics* 35 91 1965

2 — Studies on the ontogeny and significance in neonatal disease of the fibrinolysin system and of fibrin stabilizing factor *Thromb Diath haemorrh* 19 599 1968

3 Andersson L Nilsson I M & Olow B Fibrinolytic activity in man during surgery *Thromb Diath haemorrh* 7 391 1962

4 Ekelund H Hedner U & Nilsson I M Fibrinolysis in newborns *Acta Paediat Scand* 59 33 1970

5 Ganrot P O Determination of α₂-macroglobulin as trypsin protein esterase *Clin Chim Acta* 14 493 1966

6 Ganrot P O & Nilehn J E Immunochemical determination of human plasminogen Clin Chim *Acta* 22 335 1968

7 Ganrot P O & Scherstén B Serum α₂-macroglobulin concentration and its variation with age and sex *Clin Chim Acta* 15 113 1967

8 Gitlin D & Biasucci A Development of γG A γM β₁c/β₁A C1 esterase inhibitor ceruloplasmin transferrin hemopexin haptoglobin fibrinogen plasminogen α₁ antitrypsin orosomucoid β lipoprotein α₂ macroglobulin and prealbumin in the human conceptus *J Clin Invest* 48 1433 1969

9 Kretchmer N Greenberg R F & Sereni F Biochemical basis of immaturity *Ann Rev Med* 14 407 1963

10 Ludwig H Mikrozirkulationsstörungen und Diapedeseblutungen im fetalen Gehirn bei Hypoxie Bibl Gynaec vol 46 Karger Basel New York 1968

11 Nilehn J E Separation and estimation of "split products" of fibrinogen and fibrin in human serum *Thromb Diath haemorrh* 18 3 1967

12 Nilsson I M Blombäck M & von Francken I On an inherited autosomal hemorrhagic diathesis with antihemophilic globulin (AHG) deficiency and prolonged bleeding time *Acta Med Scand* 159 35 1957

13 Nilsson I M & Olow B Determination of fibrinogen and fibrinogenolytic activity *Thromb Diath haemorrh* 8 297 1962

14 — Fibrinolysis induced by streptokinase in man *Acta Chir Scand* 123 247 1962

15 Pandolfi M Nilsson I M & Martinsson G A method for determining serum inhibitors of plasminogen activation *Scand J Clin & Lab Invest* 14 138 196

16 Sereni F & Principi N The development of enzyme systems *Ped Clin N Amer* 12 51 1965

17 Sharp A A The significance of fibrinolysis *Proc Roy Soc B* 173 311 1969

18 Vahlquist B Westberg V & de las Heras M

Prothrombin and fibrinogen values in the young human fetus *Acta Soc Med Upsal 58* 281 1953
19 Zilliacus H Ottelin A M & Mattson T Blood clotting and fibrinolysis in human foetuses *Biol Neonat 10* 108 1966

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DISLOCATION OF THE PROXIMAL EPIPHYSIS OF THE HUMERUS IN NEWBORNS

Report of Two Cases and Discussion of Diagnostic Criteria

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An early diagnosis of traumatic dislocation of the proximal epiphysis of the humerus in newborns present some difficulties Traumatic dislocation of the proximal epiphysis of the humerus caused by birth injuries was mentioned by Truesdell (8) One case of extensive caudal dislocation of the epiphysis in relation to the shaft of the humerus was reported by Michel (4) Recently 3 cases were described by Mennen & Rosa (3) Traumatic injury to the epiphysis of the proximal humerus was realized roentgenologically at 15–20 days of age due to a visible callus fromation Scaglietti (5) published 53 cases of birth injuries affecting the proximal epiphysis of the humerus Apparently only few of these were diagnosed in newborns In a systematic study of newborns after breech delivery Snedecor & Wilson (7) noticed epiphyseal dislocations and periosteal stripping at various sites one of these localized to the proximal part of the humerus Blount (1) also pointed out the difficulty of early diagnosis and stressed the necessity of repeating the X ray examination after about 1 week in order to detect callus formation

Two cases of dislocation of the proximal epiphysis of the humerus in newborns are presented

CASE REPORTS

Case 1 Girl born by podalic version and rapid extraction After birth a paralysis of the left arm of Erb's type was noticed An X ray examination of the shoulder joint at this time showed no pathological changes A certain regress of the paralysis was observed on the seventh day after birth The same day a new X ray examination was made (Fig 1) A lateral displacement of the boney epiphyseal nucleus in relation to the humerus shaft was recognized as well as a calcification lateral to the proximal end of the humerus The X ray examination was interpreted as a luxation of the shoulder joint An attempt to reduce the luxation under roentgen television control was unsuccessful Therefore interposition of soft tissue obstructing reposition was considered On a new examination 3 days later a slight deformity of the contour of the left shoulder area was visible with a slight angulation at the level of the deltoid muscle attachment and a soft tissue swelling around the proximal end of the humerus could be palpated Spontaneous movements in the shoulder joint was restricted but the biceps muscle showed clear signs of activity Passive movements in the shoulder joint was normal but obviously evoked pain Therefore clinically an epiphyseolysis or epiphyseal fracture of the proximal humerus was considered A new X ray examination 4 days later showed more or less the same findings as in Fig 1 and was now interpreted as an epiphyseal injury The arm was placed on an abduction splint for 2 weeks After this spontaneous movements in the shoulder joint returned and the passive mobility was within normal range At the same time a new X ray examination revealed abundant callus round the proximal end of the humerus (Fig 2) No further treatment was given X ray examination at 6 months of age showed a normal proximal humerus (Fig 3) with normal clinical findings

Case 2 Girl born by podalic version Immediately after birth a certain angulation was seen of the left upper arm. A soft tissue swelling round the proximal end of the humerus was palpated and a partial paralysis of the Erb type was found Under careful

Prothrombin and fibrinogen values in the young human fetus *Acta Soc Med Upsal 58* 281 1953
19 Zilliacus H Ottelin A M & Mattson T Blood clotting and fibrinolysis in human foetuses *Biol Neonat 10* 108 1966

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Fig 4 Case 1 Left (injured) shoulder in inward rotation of the humerus

joints in symmetrical inward and outward rotation (with the elbows flexed to 90) The importance of the rotational position for demonstration of a dislocation between epiphysis and shaft is shown in Figs 4 and 5 On the right (normal) side the epiphyseal nucleus is lying medially to an extension of the shaft of the humerus On the left (injured) side the lateral dislocation of the epiphyseal nucleus in relation to the shaft of the humerus becomes visible

Dorsal or ventral luxation can best be excluded by axial X ray whilst a caudal luxation can be seen on frontal pictures On the other hand an extensively dislocated epiphyseal injury *without* visible nucleus can appear as a caudal luxation when the picture is taken in abduction

Where no boney nucleus in the epiphysis can be seen a swelling of the soft parts round the joint and increased distance between the proximal end of the humerus and the scapula can be a sign of bleeding in or around the joint and thereby point to an injury to the epiphysis Minimum calcium deposits can be visible after some days (Fig 1) According to previous reports even a completely normal X ray cannot with c rtainty exclude the presence of an epiphyseal injury (1 5)

(b) Infants 10–16 days of age

Clinical symptoms Swelling and deformity can still be detected whilst the reduction of function or paralysis can show considerable regress Examination of the shoulder joint can show normal range of movement but can still cause pain

X ray changes X ray examination at this stage usually show callus around the proximal end of the humerus

The clinical symptoms of an epiphyseal injury differ from a pure brachial plexus injury as regards to the visible and palpable deformation of the proximal end of the humerus The obvious tendency to rapid improvement of active function in the shoulder joint also gives reason to assume that the noticeable decrease in function is more likely to be a restriction due to pain rather than to an organic nerve injury This interpretation is supported by the fact that

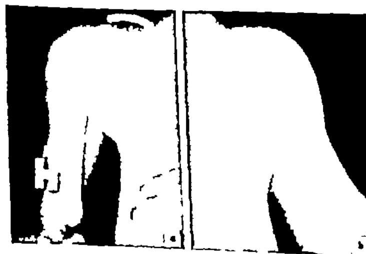


Fig 5 Case 1 Right normal (a) and left, injured (b) shoulder in outward rotation of the humerus with altered relation between epiphyseal nucleus and shaft of the humerus on injured side



Fig 1 Case 1 Left shoulder at 7th day of age Lateral displacement of bony epiphysis in relation to the shaft of the humerus Small calcification adjunct to proximal end of humerus (*arrow*)

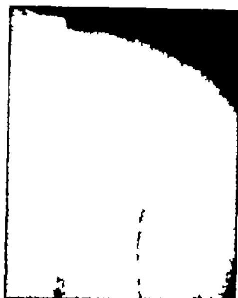


Fig 3 Case 1 Normal left shoulder joint at the age of *1* year

examination there was a passively free but clearly painful movement of the shoulder joint An X ray examination the day after birth showed a slight increase in the distance between the very small but visible epiphyseal nucleus in the proximal humerus and the scapula and a probable injury of the epiphysis was diagnosed The arm was fixed to the body A new examination ten days after birth showed a definite *regress of the paralysis as well as of the* palpable resistance round the proximal end of the humerus Passive movement in the shoulder joint still seemed to evoke some pain A new X ray showed *small amounts of callus round the proximal part of* the humerus and the boney epiphysis appeared more laterally situated than on the normal side No further treatment was given X ray examination 3 months later showed a lateral dislocation of the boney epiphysis in relation to the shaft of the humerus as well as some deformity and callus remnant round the proximal end of the humerus The shoulder joint was clinically normal except that a slight angulation of the proximal contour of the upper arm could still be seen



Fig 2 Case 1 Left shoulder at 3 weeks of age Heavy calcified callus around the proximal humerus

DISCUSSION

The two above reported cases of traumatic dislocation of the proximal epiphysis of the humerus agree for the most part with the symptomatology described in previous reports Clinical and X-ray findings which correctly interpreted can help to ensure the diagnosis at an early stage will be summarized and discussed for newborns and for infants above 10 days of age separately

(a) Newborns and infants up to 9 days of age

Clinical symptoms Reduced spontaneous movements pseudo paralysis or paralysis of the arm *may be found These are often of the Erb type* The arm often lies inwardly rotated Visible deformity and/or palpable swelling round the proximal end of the *humerus but with no signs* of humerus shaft fracture or noticeable luxation in the humero scapular joint points to an epiphyseal injury *Passive movements in the* shoulder joint are obviously painful

X ray changes When there is a visible boney nucleus in the epiphysis (*present in about 50* of newborns Garn *et al* (2)) lateral dislocation in relation to the shaft of the humerus and increased distance *to the scapula* can be seen X ray pictures must be taken of both shoulder

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SERUM HAPTOGLOBIN LEVEL IN DISSEMINATED MALIGNANT DISEASES IN CHILDREN

PETER JOHAN MOE

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Haptoglobin is an α glycoprotein which binds hemoglobin The plasma concentration of this glycoprotein is raised in most diseases associated with destruction of tissue and is decreased in hemolytic disorders In adults suffering from cancer both high and normal values have been found (4)

The main purpose of this investigation was to assess whether determination of serum haptoglobin could be of differential diagnostic aid in disseminated malignant diseases In addition it was of interest to see whether different types of drugs influence the haptoglobin level in malignant diseases and whether serum haptoglobin determination could be useful in assessing the activity of the malignant disease

In addition to quantitative variations haptoglobin also exhibits qualitative variations It has been suggested that a certain haptoglobin type possible may be of significance in leukemia (5) Therefore attempts were made in this study to see if certain haptoglobin types were prevalent in disseminated malignant diseases particularly in childhood leukemia

MATERIAL AND METHODS

Analyses were performed on serum samples from a total of 140 children 58 cases of leukemia 18 cases of other disseminated malignant diseases (8 cases of neuroblastoma 9 cases of malignant lymphoma and 1 case of Ewing sarcoma) 9 cases of severe infections 20 cases of benign blood diseases and 35 healthy children Serum samples from 27 of the leukemic cases were from other hospitals

Most of the patients from the Children's Hospital have been followed on an outpatient basis after discharge from the hospital Samples were obtained from most of those cases both prior to and during different types of therapy All studies have been performed during the last 3 years

All but one of the patients with malignant diseases and all the 35 healthy children were aged 1 to 13 years

The drug therapy was divided into the following three types

1 Prednisone therapy
2 Combined prednisone and cytostatic therapy
3 Cytostatic therapy

The cytostatic drugs used were 6-mercaptopurin aminopterin cyclophosphamide vincristin and Natulan®

Serum haptoglobin was measured spectrophotometrically by the method of Tarukoski (8) Duplicate determination on 30 random samples (mean haptoglobin of 137 mg per 100 ml) gave a mean error of the method of 3 7 mg per 100 ml The error was relatively greater when only small amounts of haptoglobin was present

Haptoglobin phenotypes were determined by starch electrophoresis by the method of Smithies (7)

RESULTS

Table 1 shows that there is no significant difference in mean haptoglobin level in 40 untreated cases of leukemia and 17 untreated cases of other disseminated malignant disease Nor was there significant difference between the leukemic cases and 9 children with severe

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These determinations were made by Halldor Lie M.D. Institute of Forensic Medicine University of Oslo

Sever (6) among 1 100 obstetric paralyses of the upper extremity denied the occurrence of epiphyseal injury of the proximal humerus

The essential factor when estimating the X ray pictures is that the lateral projection of the epiphyseal nucleus is not interpretated as a luxation in the shoulder joint but as a disloca tion of the epiphysis

In analogy with *epiphyseal* injuries in other long bones this injury can be accompanied by a tearing off of a periosteal bone flap from the metaphysis periosteal stripping (7) This can be visible on X ray as a small calcium deposit (Fig 1) and thereby gives an early indication of traumatic epiphyseal injury before a larger callus mass becomes visible

CONCLUSION

1 Traumatic injury of the proximal humerus epiphysis especially after delivery in breech position or podalic version should be taken into account in newborn children showing signs of *restricted spontaneous function in the shoulder* joint or a picture resembling brachial plexus injury

2 Clinically alteration of the contour of the shoulder area can be seen movement in the shoulder joint can be painful and swelling round the proximal end of the humerus can be palpa ted without signs of fracture of the humerus shaft or luxation of the shoulder joint

3 A diagnosis by means of X ray can be made with certain probability on newborns and up to 9 days of age provided a boney epiphyse al nucleus is present and pictures with outward rotation of the upper arm are taken symmetric ally from both sides Even very small calcium deposits in the soft parts round the proximal end of the humerus can indicate an injury to the epiphysis (periosteal stripping) Negative X ray findings do not exclude epiphyseal injury

4 X ray examinations should be repeated between 10–14 days of age on all children with one or several of above mentioned criteria as callus round the proximal end of the humerus can usually be seen in a traumatic epiphyseal injury

SUMMARY

Two cases of traumatic dislocation of the prox imal humerus epiphysis in newborns are re ported *Clinical symptoms for early diagnosis* and differentiation from pure brachial plexus injuries are described Stress is lied on the local *findings around the shoulder joint and the im* portance of taking X ray pictures in suitable symmetrical positions for demonstration of minor dislocations of the epiphyseal nucleus

REFERENCES

1 Blount W P *Fractures in children* Williams & Wilkins Baltimore 1955
2 Garn S M Rohmann C G & Silverman F N Radiographic standards for postnatal ossification and tooth calcification *Med Radiogr Photogr 43* 45 1967
3 Mennitti D & Rossi A I Distacchi epifisari Obstetrici *Arch Putti 21* 192 1966
4 Michel L Le decollement obstetrical de l'epi physe supérieure de l'humerus *Rev Chir Orthop 24* 201 1937
5 Scaglietti O The obstetrical shoulder trauma *Surg Gynec Obstet 66* 868 1938
6 Sever J W Obstetric paralysis Report of eleven hundred cases *JAMA 85* 1862 1925
7 Snedecor S T & Wilson H B Some obstetrical injuries to the long bones *J Bone Joint Surg 31A* 378 1949
8 Truesdell E D *Birth fractures and epiphyseal dis locations* Paul B Hoeber Inc New York 1917

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Table 1 shows that there is no significant difference in mean haptoglobin level in 40 untreated cases of leukemia and 17 untreated cases of other disseminated malignant disease Nor was there significant difference between the leukemic cases and 9 children with severe

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Table 1. *Serum haptoglobin concentration in untreated children with leukemia compared with some other diseases*

Group		No. of cases	Mean haptoglobin mg per 100 ml	S.D.	Range	t Test groups	
I	Leukemia	40	254	111	71–474	I/II	Not significant $p > 0.1$
II	Other malignant dis.	17	235	62	115–300		
III	Severe infections	9	279	31	244–309	I/III	Not significant $p > 0.1$
IV	Reticuloendotheliosis	7	127		33–248		
	Mononucleosis	6	30		0–112		
	Osteopetrosis	2	0		0		
	Hemolytic anemia	5	11		0–30		
V	Control group	35	69	20	32–117	I/V & II/V	Significant $p < 0.01$

infections. All three groups had significantly higher mean haptoglobin levels than the controls and the cases of non-malignant diseases.

Table 2 shows that high mean haptoglobin level also existed during prednisone therapy.

The mean haptoglobin level in the leukemic cases receiving cytostatic therapy was markedly lower than those observed in untreated cases and in cases receiving prednisone therapy.

Table 3 shows serum haptoglobin values prior to therapy and during prednisone and cytostatic therapy in 12 leukemic cases followed closely by the author for 3 months to 3 years. They represent almost all the cases in Table 2 in whom more than one analysis was performed during therapy. Cases were considered to be in complete remission when they had no clinical or hematological signs of their leukemia. The table shows that high haptoglobin values also existed during prednisone therapy in cases considered to be in complete remission. Low values were found both while the children received 6-mercaptopurin (mean value of 60 mg per 100 ml) and aminopterin (mean value of 63 mg per 100 ml) therapy. The number of cases is small, but there was practically no overlapping between the values during prednisone and cytostatic therapy, regardless of presence or absence of remission.

There was a high incidence of haptoglobin values below 30 mg per 100 ml in leukemic cases during cytostatic therapy (Table 3) while none of 35 healthy children had such low values. However, no significant difference was found between the two groups (Table 2).

Both high and low values were seen in leu-

Table 2. *Serum haptoglobin concentration in children with malignant diseases prior to and during different types of therapy*

Group	Therapy	No. of cases	No. of tests	Mean haptoglobin mg per 100 ml	Range	t Test groups	
I Leukemia	(*a*) Prior to therapy	40	40	254	71–474	Ia/Ib	Not significant $p > 0.1$
	(*b*) Prednisone	14	21	226	78–570	Ib/Id	Significant $p < 0.01$
	(*c*) Predn. + Cytostatic	25	56	143[a]	0–347		
	(*d*) Cytostatic	14	40	68	0–145	Ia/Id	Significant $p < 0.01$
II Other malign. dis.	(*a*) Prior to therapy	17	17	235	107–300		
	(*b*) Prednisone	3	3	242	150–295		
	(*c*) Cytostatic	12	42	109[a]	0–300	IIa/IId	Significant $p < 0.01$
V Controls		35	35	69	32–117	Id/V	Not significant $p > 0.1$

[a] Mean and t test are calculated from the mean of each case.

Table 3 *Serum haptoglobin concentration (in mg per 100 ml) in 12 children with leukemia prior to therapy and during prednisone and cytostatic therapy*

Patient	Prior to therapy	Prednisone therapy	Cytostatic therapy	
			6-Mercaptopurin	Aminopterin
By	136		*69 28 18 64*	
Fo	189		*28*	
Gr	315	217 336		
Ha	293	*227*	*60 69 29*	
Ho	308	07 225	*11 53 17 20 29 33*	*14 114 88*
Iv	245	*150*	20	20
Jo	372	570 255 *371*		
Kr	212			70
Li	375	252	*126*	*47 137*
Mi	136	*117 372 136*	134	*52 70*
Mo	278		*79*	104
Se	233	*78 150*	*40 10*	*24 30 20 0 14*
Mean	258	230	60	63

Samples during the combined therapy with prednisone and cytostatics are not included in this table
Figures in italics refer to samples during clinical and hematological remission
Mean calculated from the mean of each case

kemic cases during the combined therapy with prednisone and cytostatics This group is however to complex for further evaluation

The mean haptoglobin level during prednisone therapy in the group of other malignant diseases was significantly lower than that observed in untreated cases

Haptoglobin phenotype studies were performed in a total of 52 cases of malignant diseases including 41 cases of leukemia The distribution of th three groups corresponded to the distribution in a normal Norwegian population (Table 4)

DISCUSSION

Serum haptoglobin level seems to be of little differential diagnostic aid in malignancy in childhood as high values are seen in leukemia other disseminated malignant diseases and severe infections Low haptoglobin level seems however to be unlikely in disseminated malignant diseases and is more compatible with a benign type of blood disorder

Hyperhaptoglobinemia during the prednisone therapy may be due to lack of remission However high values were also found in leukemic cases in complete remission It has been shown that corticosteroids stimulate the production of haptoglobin (2 3 6)

The *low level in leukemic* cases receiving cytostatic therapy may be due to the fact that most of the leukemic cases were in complete remission However remission alone can hardly explain the pathological low values observed in this group It is difficult to evaluate the isolated effect of a cytostatic agent as one cannot give cytostatic therapy to a healthy child and studies on the influence on haptoglobin of cytostatic therapy have not been reported

It has not be.n possible to find comparable data in the literature Turovska (9) and Peacock (5) reported the results of therapy in a

Table 4 *Distribution of the haptoglobin groups in dissem rated malignant diseases in children*

Disease	No of cases of haptoglobin type			Total no of cases
	1 1	1 2	2 2	
Leukemia	5	21	15	41
Neuroblastoma	0	3	2	5
Malignant lymphoma	2	2	2	6
Total no of cases	7	26	19	52
Normal distribution*	132	462	406	1 000

Fleischer & Lundevall (1)

few cases of different types of leukemia in adults But information as to the type of therapy is not given

Peacock suggested in 1966 (5) that the gene for haptoglobin protein type 2 confers resistance to leukemia on the holders to such an extent that the relative risk of leukemia among homozygous 1–1 as compared with subjects 2–2 is approximately fourfold Among his 36 cases of lymphoblastic leukemia there were 30 6 % haptoglobin type 1–1 and only 16 7% type 2–2 This predominance was not found among our material of 41 studied cases of childhood leukemia the ratio type 1–1 to 2–2 was 1 3 the same as in a normal Norwegian population Further studies seem indicated before any conclusions can be drawn as to the possible etiological significance of the serum haptoglobin type

SUMMARY AND CONCLUSIONS

Serum haptoglobin studies have been performed in a total of 140 children 76 cases of disseminated malignant diseases 9 cases of severe infections 20 cases of benign blood disorders and 35 healthy controls

Hyperhaptoglobinemia was found in a large proportion of 40 untreated cases of leukemia and 17 cases of other malignant diseases as well as in 9 cases of severe infections Serum haptoglobin seems therefore to be of little differential diagnostic aid in malignancy in children Low values are however unlikely in disseminated malignant diseases in children and more compatible with a benign type of blood disorder

The serum haptoglobin level seems to be dependent not only on the activity of the malignant disease but also on the type of therapy The haptoglobin level is therefore of little practical use as a parameter of the activity of a malignant process in childhood

A normal distribution of serum haptoglobin type was found in 52 cases of disseminated malignant diseases including 41 cases of acute leukemia

REFERENCES

1 Fleischer R & Lundevall J Inheritance of serum groups *Proc 6th Congr European Soc Haemat* Copenhagen 1957 S Karger Basel 1958
2 Galatius Jensen F *The haptoglobins A genetic study* Dansk Videnskabs Forlag København 1960
3 Jayle M F & Boussier G Les seromucoides du sang leur relations avec les mucoproteines de la substance fundamentale de tissu conjonctif *Expos Ann Biochim Med 17* 757 1955
4 Nyman M Serum haptoglobin Methodological and clinical studies *Scand J Clin & Lab Invest* Suppl 39 1959
5 Peacock A C Serum haptoglobin type and leukemia An association with possible etiological significance *J Nat Cancer Inst 36* 631 1966
6 Smith C H *Blood diseases of infancy and childhood* C V Mosby Company St Louis 1966 2nd ed
7 Smithies O An improved procedure for starch gel electrophoresis in the serum proteins of normal individuals *Biochem J 71* 585 1959
8 Tarukoski P H Quantitative spectrophotometric determination of haptoglobin *Scand J Clin & Lab Invest 18* 90 1966
9 Turovskh M G The effect of therapy on the haptoglobin level in leukemic patients *Pre Le Karski 4* 381 1967

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BLADDER STONE DISEASE OF CHILDHOOD

II *A Clinico-Pathological Study*[1]

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Bladder stone disease of childhood continues to be a pediatric problem in South East Asia (1 4 23 35 40 48) Its patchy distribution in Thailand and India suggests the possibility of nutritional factors in its causation McCarrison (35) on the basis of his experimental work in rats emphasized the importance of Vitamin A and infection in the genesis of bladder stone However it is well known that bladder stones can form in uninfected urine (34) In recent epidemiological studies in Thailand bladder stones were found in much larger number in the village population Increased excretion of Calcium and the presence of oxalate crystals in urine was a frequent finding in this population as compared to the urban population of Thailand (24 49) On the other hand in India Andersen *et al* (1) failed to find any significant hypercalciuria in their patients under the age of 20 years Thus so far the etiology and pathogenesis of this condition remains obscure Further the relationship of this condition with the more universally distributed renal calculus has also not been clearly defined

A clinico-pathological study of 61 cases of bladder stone in childhood was carried out to elucidate the role of factors believed to be responsible in the pathogenesis of this malady

MATERIAL AND METHODS

A *Clinical Study*

Clinical examination was undertaken with a special emphasis on the detection of any nutritional disorder Any obstructive uropathy at the time of admission or at operation was recorded

Hemoglobin estimation, packed cell volume urinalysis and plain X ray of the entire urinary tract were undertaken Urine samples for routine and culture studies were obtained directly from the urinary bladder at the time of operation. Intravenous pyelography was undertaken in 16 cases

B *Biochemical Study*

The following biochemical estimations were done in blood and urine

(a) *Blood chemistry*

1 Serum Calcium (32)
2. S roomucoids (34)
3 Plasma Vitamin A and carotene using carr price reagent as given by Varley (50)
4 Plasma inorganic phosphorus using stannous chloride (37)
5 Blood urea (32)
6 Plasma protein total by Biuret method (32) and differential by paper electrophoresis (25)

(b) *Urine chemistry*

1 Estimation of calcium before and after keeping the patient for 4 days on 150 mgms calcium diet, by McCrudden s method as quoted by Varley (50)
2 Oxalate (16) before and after tryptophan load
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5 Xanthurenic Acid (53) after tryptophan load

[1] Partly supported by a grant from the Indian Council of Medical Research

few cases of different types of leukemia in adults But information as to the type of therapy is not given

Peacock suggested in 1966 (5) that the gene for haptoglobin protein type 2 confers resistance to leukemia on the holders to such an extent that the relative risk of leukemia among homozygous 1–1 as compared with subjects 2–2 is approximately fourfold Among his 36 cases of lymphoblastic leukemia there were 30 6 haptoglobin type 1–1 and only 16 7* type 2–2 This predominance was not found among our material of 41 studied cases of childhood leukemia, the ratio type 1–1 to 2–2 was 1 3 the same as in a normal Norwegian population Further studies seem indicated before any conclusions can be drawn as to the possible etiological significance of the serum haptoglobin type

SUMMARY AND CONCLUSIONS

Serum haptoglobin studies have been performed in a total of 140 children 76 cases of disseminated malignant diseases 9 cases of severe infections 20 cases of benign blood disorders and 35 healthy controls

Hyperhaptoglobinemia was found in a large proportion of 40 untreated cases of leukemia and 17 cases of other malignant diseases as well as in 9 cases of severe infections Serum haptoglobin seems therefore to be of little differential diagnostic aid in malignancy in children Low values are however unlikely in disseminated malignant diseases in children and more compatible with a benign type of blood disorder

The serum haptoglobin level seems to be dependent not only on the activty of the malignant disease but also on the type of therapy The haptoglobin level is therefore of little practical use as a parameter of the activity of a malignant process in childhood

A normal distribution of serum haptoglobin type was found in 52 cases of disseminated malignant diseases including 41 cases of acute leukemia

REFERENCES

1 Fleischer R & Lundevall J Inheritance of serum groups *Proc 6th Congr European Soc Haemat* Copenhagen 1957 S Karger Basel 1958
2 Galatius Jensen F *The haptoglobins A genetical study* Dansk Videnskabs Forlag København 1960
3 Jayle M F & Boussier G Les seromucoides du sang leur relations avec les mucoproteines de la substance fundamentale de tissu conjonctif *Expos Ann Biochim Med* 17 757 1955
4 Nyman M Serum haptoglobin Methodological and clinical studies *Scand J Clin & Lab Invest* Suppl 39 1959
5 Peacock A C Serum haptoglobin type and leukemia An association with possible etiological significance *J Nat Cancer Inst* 36 631 1966
6 Smith C H *Blood diseases of infancy and childhood* C V Mosby Company St Louis 1966 2nd ed
7 Smithies O An improved procedure for starch gel electrophoresis in the serum proteins of normal individuals *Biochem J* 71 585 1959
8 Tarukoski P H Quantitative spectrophotometric determination of haptoglobin *Scand J Clin & Lab Invest* 18 90 1966
9 Turovska M G The effect of therapy on the haptoglobin level in leukemic patients *Pre Lekarski* 4 381 1967

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Key words Haptoglobin malignant disease

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BLADDER STONE DISEASE OF CHILDHOOD

II *A Clinico Pathological Study*[1]

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Bladder stone disease of childhood continues to be a pediatric problem in South East Asia (1 4 23 35 40 48) Its patchy distribution in Thailand and India suggests the possibility of nutritional factors in its causation McCarrison (35) on the basis of his experimental work in rats emphasized the importance of Vitamin A and infection in the genesis of bladder stone *However it is well known that* bladder stones can form in uninfected urine (34) In recent epidemiological studies in Thailand bladder stones were found in much larger number in the village population Increased excretion of Calcium and the presence of oxalate crystals in urine was a frequent finding in this population as compared to the urban population of Thailand (24 49) On the other hand in India Andersen *et al* (1) failed to find any significant hypercalcemia in their patients under the age of 20 years Thus so far the etiology and pathogenesis of this condition remains obscure Further the relationship of this condition with the more universally distributed renal calculus has also not been clearly defined

A clinico-pathological study of 61 cases of bladder stone in childhood was carried out to elucidate the role of factors believed to be responsible in the pathogenesis of this malady

[1] Partly supported by a grant from the Indian Council of Medical Research.

MATERIAL AND METHODS

A *Clinical Study*

Clinical examination was undertaken with a special emphasis on the detection of any nutritional disorder Any obstructive uropathy at the time of admission or at operation was recorded

Hemoglobin estimation packed cell volume urinalysis and plain X ray of the entire urinary tract were undertaken Urine samples for routine and culture studies were obtained directly from the urinary bladder at the time of operation Intravenous pyelography was undertaken in 16 cases

B *Biochemical Study*

The following biochemical estimations were done in blood and urine

(a) *Blood chemistry*

1 Serum Calcium (32)
2 S ionocalcium (54)
3 Plasma Vitamin A and carotene using carr price reagent as given by Varley (50)
4 Plasma inorganic phosphorus using stannous chloride (32)
5 Blood urea (32)
6 Plasma protein total by Biuret method (32) and differential by paper electrophoresis (25)

(b) *Urine chemistry*

1 Estimation of calcium before and after keeping the patient for 4 days on 150 mgms calcium diet, by McCrudden's method as quoted by Varley (50)
2 Oxalate (16) before and after tryptophan load
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4 Nyman M Serum haptoglobin Methodological and clinical studies *Scand J Clin & Lab Invest* Suppl 39 1959
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6 Smith C H *Blood diseases of infancy and childhood* C V Mosby Company St Louis 1966 2nd ed
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Table 2 *Statistical analysis of data on blood chemistry of bladd r stone cases*

	Calcium (mg per 100 ml)		Vitamin A corrected values (μg per 100 ml)		Keromotocords (mg per 100 ml)	
	Patients	Control	Patients	Control	Patients	Control
0–4 years						
Mean	9 57 (22)	8 225 (4)	9 953 (15)	11 625 (4)	17 994 (16)	11 875 (4)
S D	0 884	0 436	4 125	3 493	5 367	1 473
Significant	Not		Not		Significant value of p 0 05	
5–9 years						
Mean	9 53 (18)	9 47 (10)	12 03 (16)	18 74 (9)	17 82 (14)	12.758 (5)
S D	0 888	0 473	3 834	3 187	7 463	2.565
Significant	Not		Highly significant alue of p 0 001		Not	
10–14 years						
Mean	10 116 (6)	9 83 (3)	10 62 (6)	14 80 (5)	15.204 (5)	12.12 (2)
S D	0 443	0 711	5 514	4 906	4 416	0 686
Significant	Not		Not		Not	

Values in parenthesis indicate the number of cases studied

normal limits and were very much similar in patients and controls

The level of proteins tended to be high in stone cases The values were statistically much higher in the age group 5–9 years with marked increase in beta globulins

The levels of blood urea ranged from 20 to 86 mg/100 ml with more than 40 mg/100 ml in 9 patients

(c) Urinalysis

The results of chemical microscopical and bacteriological analysis of urine are summarized in Table 4

The pH of urine in the majority of the cases was acidic Protein was present in 26 of the 37 cases investigated The number of pus cells and red blood cells varied from case to case and did not seem to bear any relation to each

Table 3 *Statistical analysis of data on blood chemistry of bladder stone cases*

Age group	Phosphorus (mg per 100 ml)		Proteins (g per 100 ml) Total		Albumin		Globulin	
	Patients	*Control*	*Patients*	*Control*	Patients	Control	Patients	Control
0–4 years								
Mean	5 33 (4)	5 938 (4)	8.246 (18)	7 835 (4)	3 836 (17)	3 655 (4)	4 458 (17)	4 18 (4)
S D	1 233	0 170	0 899	0 491	0 868	0 237	0 369	0 240
Significant	Not		Not		Not		Not	
5–9 years								
Mean	4 663 (6)	5 159 (9)	9 073 (14)	7 904 (7)	4 393 (14)	3 472 (7)	4 77 (14)	4 34 (7)
S D	1 039	0 390	1 049	0 669	0 897	0 609	1 192	0 665
Significant	Not		Highly significant value of p < 0 02		Significant value of p < 0 05		Not	
10 14 years								
Mean	5 277 (4)	4 977 (3)	10 87 (6)	9 0233 (3)	4 678 (6)	4 15 (3)	5 625 (6)	4 873 (3)
S D	0 996	0 349	1 637	0 826	1 144	0 078	1 635	0 769
Significant	Y ot		Not		Not		Not	

Values in parentheses indicate the number of cases studied

(c) *Morphological studies*

This included study of bladder biopsies and bladder stones

RESULTS

A Clinical Studies

(a) *History*

80 3% of the cases came from very poor families with an income of less than 150 rupees and the rest had an income ranging from 150 to 400 rupees per month

The staple cereal consumed by the patients was wheat with or without a supplement of jowar bajara black gram or rarely rice Milk eggs and meat did not form a significant part of the diet The intake of leafy vegetables was generally low

In none of these cases a family history of bladder stone was available

(b) *Symptoms and signs*

The symptoms which brought the patients to the hospital are given in Table 1 The duration of symptoms ranged from 6 days to 8 years In two cases where the stones were milked out of urethra the duration of symptoms was only 3 and 4 hours respectively

The physical signs were few and non-specific The children showed in general some evidence of growth retardation although no specific signs of deficiency disease were noticeable Special mention need be made of the absence of ocular signs of Vitamin A deficiency such as Bitot s spots and Keratomalacia

Except for one case with phimosis and another with bladder neck obstruction, there was no evidence of obstruction in the lower urinary tract

B Laboratory Studies

(a) *Hemoglobin packed cell volume and absolute values of blood*

All the cases had some degree of iron deficiency anemia as suggested by low mean corpuscular hemoglobin concentration (MCHC) The anemia was slightly more marked in 0-4 years age group as compared to control children The hemoglobin and MCHC values in the patients were $10\ 30 \pm 1\ 36$ g/100 ml and $28\ 82 \pm 1\ 19$ g/100 ml respectively The values in control children were $11\ 39 \pm 1\ 19$ g/100 ml and $30\ 21 \pm 2\ 67$ g/100 ml In 5–9 years and 10–14 years age group there was no difference from the control children of the poor population

(b) *Blood chemistry*

A statistical analysis of blood constituents is given in Tables 2 and 3 The serum calcium values were similar in the patients and controls

The plasma Vitamin A (corrected) values ranged between 3 0 to 19 0 μg/100 ml in the patients and 8 0 to 29 0 μg/100 ml in controls In the 5–9 years age group the values were significantly lower in patients than in controls

The value of seromucoids were higher in patients than in controls and showed a statistical significance in the age group 0–4 years

The plasma phosphorus levels were within

Table 1 *Frequency of symptoms of 61 cases with bladder stone*

	Pulling & rubbing of penis in 56 male patients	Pain during micturition	Increased frequency of micturition	Dribbling	Hematuria	Burning micturition	Pain in supra pubic region	Retention of urine
No of cases	52	44	29	22	19	18	12	2
Percentage of the total	92 67 of the 56 male patients	72 13	47 54	36 06	31 15	29 51	24 59	3 28

Table 5 *Statistical analysis of data of urine chemistry in bladder stone cases*

Age group	Calcium (mg per 24 hours) Patients	Control	Oxalate (mg per 24 hours) Patients	Control	Xanthurenic acid (mg per 24 hours) Patients	Control	Uromucoids (mg per 24 hours) Patients	Control
0–4 years								
Mean	63 70 (8)	34 20 (4)	12 47 (9)	12 09 (4)	22 26 (4)	19 93 (4)	99 50 (6)	55 47 (4)
S D	43 389	20 10	3 975	3 464	5 03	3 098	21 074	24 88
Significant	Not		Not		Not		Highly significant value of *p* between 0 02 and 0 01	
5–9 years								
Mean	63 85 (11)	41 31 (6)	17.29 (12)	13 10 (6)	22.54 (6)	18 89 (5)	119 02 (9)	97 74 (6)
S D	42 190	19 391	7 470	5 779	9 322	4 159	78 930	32 404
Significant	Not		Not		Not		Not	
10–14 years								
Mean	56 35 (4)	34.29 (2)	20 87 (4)	13 50 (2)	24 27 (3)	18 60 (2)	171 46 (4)	101 54 (2)
S D	54 123	10 909	6 580	0 990	5 00	8 025	48 270	3 768
Significant	Not		Not		Not		Not	

Value in parentheses indicate the number of cases studied

0–4 years age group were highly significant when compared with controls In the other two age groups though the values were higher in patients than in controls but due to wider scatter of the valu s they were not statistically significant Thus it would be observed that generally the patients with bladder stone excreted more calcium and more uromucoids than the controls children

Radiological examination of patients

Plain X ray of the abdomen including the entire urinary tract was done in each case In none of the patients evidence of renal or ureteral stone was present Intravenous pyelography was carried out in 16 patients The findings are recorded in Table 6 31 25% of the patients had no abnormality 68 75 % of the patients had either hydroureter or hydronephrosis or both The blood urea was highest in patients having bilateral hydroureter and hydronephrosis It was interesting to note that the degree of proteinuria did not correspond with the radiological changes in the urinary tract

Table 6 *Summary table of intravenous pyelography in 16 cases with bladder stone*

	Hydronephrosis Unilateral	Bilateral	Hydroureter Unilateral	Bilateral	Hydronephrosis cum hydroureter Unilateral	Bilateral	No abnormality
Number of cases	1	NIL	NIL	2	6	2	5
Percentage of the total number studied (16)	6.25	—	—	12 5	37 5	12 5	31 25
Range of blood urea mg per 100 ml	40	—	—	37 & 44	26 to 40	56 & 86	22 to 90
Mean blood urea mg per 100 ml	—	—	—	40 5	3 66	71	36

Table 4 *Summary table of urinalysis of 44 cases with bladder stone*

Investigation	Total no of cases studied	No of positive cases in each subgroup of investigation	Culture +VE cases	Culture −VE cases
pH	39			
4 to 4 9		1	1	—
5 to 5 9		18	2	16
6 to 6 9		14	4	10
7 to 8 0		6	3	3
Protein	37			
NIL		11 (NIL)	1	10
Trace to +		20 (9)	5	15
+ ± to + +		6 (4)	3	3
Pus cells	38			
Less than 5 per HPF		24 (6)	1	17
6 to 15 per HPF		9 (5)	1	3
16 to 30 per HPF		2 (1)	NIL	1
More than 30 per HPF		3 (1)	2	NIL
Crystals	38			
Oxalates		1	NIL	1
Phosphates		4	NIL	4
Uric acid & urates		3	NIL	3
Culture	43		13	30

The numbers within parentheses are cases with more than 10 RBC per HPF indicative of hemorrhage
HPF − high power field

other or to the presence or absence of infection Of the crystals triple phosphates were present in four cases, uric acid and urates in 3 cases and calcium oxalate in one case Except in one case where amorphous urates were + + + in none of the cases crystalluria was present to any significant extent

Out of 43 urine specimens cultured 30 did not reveal any growth In the remaining 13 specimens *proteus* was isolated in 4 *Escherichia coli* in 3 *Klebsiella* in 2, *Staphylococcus pyogenes* in 2 *Pseudomonas pyocyaneus* in 1 and *Staphylococcus saprophyticus* in 1

(d) Urine chemistry

The values of creatinine were 0 23 ± 0 065 g/24 hours in the patients and 0 24 ± 0 033 g/24 hours in controls and were statistically not significant

The results of calcium, oxalate xanthurenic acid and uromucoids are given in Table 5

The calcium excretion before and after 150 mg of calcium diet failed to show any significant variation Though the mean values in patients were 1 ¹/ to 2 times that of similar values in the controls, statistically the difference was not significant due to great variation in the values in the patients There was no case of hypercalciuria

The twenty four hours oxalate excretion in patients before and after tryptophan load failed to show any significant variation between the three age groups and the controls

The excretion of xanthurenic acid in majority of the patients ranged from 15 92 to 26 74 mg/24 hours However in two patients in the age group of 5–9 years the values were 29 4 mg and 36 4 mg/24 hours These patients also tended to excrete a little more oxalate after tryptophan load than before the administration of the amino acid The possibility of Vitamin B_6 deficiency could not be ruled out in these patients However statistically the values in all the three age groups were not statistically significantly different from those of the controls

The 24 hours urinary mucoprotein levels in patients were 129 99 ± 49 408 mg (controls 83 25 ± 20 33 mg) The values in patients of



Fig. 2. Cut surface of type A stone. A central large nucleus is surrounded by a thin zone of well or granted portion formed by laminations 3

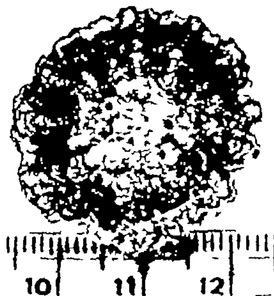


Fig. 4. Cut surface of a type C stone. A large mass of centrally located nuclei is encircled by highly scalloped laminations.

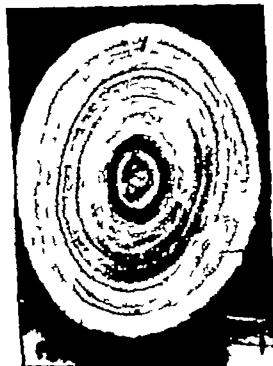


Fig. 3. Cut surface of a typical type B stone to show the regular pattern of laminations.

der 20 years of age excreted over 200 mg calcium in 24 hours.

About 0.5 to 18.6 of the cases of renal lithiasis are reported to be due to hyperparathyroidism. Niebel & Riordan (39) could find only three cases of bladder stone due to hyperparathyroidism in the world literature including one case reported by them. However none of these cases was a child. Sanong Unakul has never found a case of hyperparathyroidism related to bladder stone.

In the present series no case had a 24 hours urine calcium of 200 mg and in 83.3 the values were less than even 100 mg. The calcium metabolism was not evidently disturbed in these patients is again clear from the serum calcium values which were always less than 11.0 mg/100 ml and in no way different from the controls.

Hypophosphatemia has not been seen in the present or the earlier series reported (4, 41).

It is therefore evident that wheras disturbance in calcium metabolism are well recognized and frequently observed in renal lithiasis



Fig 1 Histologic section of the urinary bladder stained with hematoxylin and eosin (H & E) The squamoid change in the mucosa with parakeratotic cells on its luminal surface can be made out × 830

C *Morphological studies*

(a) Bladder biopsies

The changes whenever present were essentially confined to the mucosa and the underlying lamina propria The lining epithelium of the mucosa was stratified and showed two types of cells, the dark and clear cells (4) The former predominated in 13 biopsies the later in 4 and mixed type of epithelium was observed in 13 biopsies In another two biopsies the ulceration was so extensive that the cell type could not be characterized It may be mentioned that the dark cells contain large amounts of periodic acid Schiff (PAS) positive material which also forms the greater part of the matrix of stones There was no relationship between the presence of infection or presence and degree of inflammation and the predominance of any epithelial cell type in the mucosa However the dark staining cells were seen most frequently with type B stone Severe grades of ulceration of the epithelium and edema and inflammation of the lamina propria were conspicuous with the spiky type C stones

In this series in only one biopsy an area of squamoid change without keratinization was seen (Fig 1) However Vitamin A level in this patient was 11 5 μg/100 ml In several other cases the Vitamin A levels were lower and yet the epithelium did not show such a change

The stones were classified into three chief types viz A B and C (Figs 2–4) as reported earlier (4) The type of stone seen in the three age groups is given in the following table

Stone	Age Group 0–4	5 9	10–14	All Ages
A	3	–	–	3
B	28	15	5	48
C	–	8	2	10

Regarding the relationship of type of stone with the presence of infection urine culture was positive in only 30% of the cases The positive urine culture was more often associated with type C stones

The microscopic study of the stones showed features essentially similar to those reported earlier (4 41)

DISCUSSION

Calcium and phosphorus

Of the urinary mineral abnormalities hypercalciuria has been found to be the most definitive abnormality in renal lithiasis (7 19 29 37 38) It is however not present in all nor even in majority of the cases Andersen *et al* (1) reported urinary calcium levels of over 300 mg/day in 8 7% of their bladder stone cases However none of their cases un

Table 7 Differences between renal lithiasis and bladder stone disease of childhood

Parameter	Renal lithiasis	Bladder stone disease of childhood
1 Geographical distribution	World wide	Developing countries Viz South East Asia India Pakistan Southern China, Middle East and North Africa
2 Peak periods of incidence	20 to 50 years	0 to 10 years
3 Incidence by sex Males to females ratio	6 1	33 6 1
4 Socio-economic strata	All strata of society with a slightly greater predominance in the well to-do	Poor population The condition gradually disappears with general economic and nutritional improvement
5 Recurrence	Not infrequent	Rare
6 History of renal colic	Common	Practically not known
7 Hyperparathyroidism	Reported in about 1 % of cases	Not reported
8 Hypercalciuria	Present in fairly large number of cases	Not reported

Thus renal lithiasis and bladder stone disease of childhood are distinct clinico-pathological disorders with a common gross clinical manifestation of stone formation

uronucoid levels were high in the absence of changes in the kidneys It need be investigated whether the bladder urothelium of stone cases of childhood contributes some of the Andersen Maclagan material and accounts for high levels of uromucoids

Vitamin A deficiency

Erickson & Feldman (18) found defective dark adaptation in 91 of their 61 cases of urolithiasis However Aron quoted by Elliot (17) remarked that it takes several months for the Vitamin A body reserves to get exhausted to be reflected in low plasma Vitamin A levels Despite the absence of Vitamin A deficiency in United States renal lithiasis is fairly common in parts of that country Conway & coworkers (14) stated that hypovitaminosis A and hypervitaminosis D were rarities in England and yet many cases of urolithiasis were seen there In Thailand bladder stone disease is fairly common in North East Thailand even though Vitamin A deficiency is seen in all parts of that country (Uthai & Bacobo as quoted by Halstead (23)) In India Vitamin A deficiency is seen in its severest forms in the South where bladder stone is relatively infrequent as compared to Northern India (13 42 43)

Keratinizing metaplasia and subsequent stone formation is seen in rats and guinea pigs on Vitamin A deficient diets (27 35 36 45) This type of metaplastic change in the bladder urothelium in children with bladder stone has not been observed either in the present series or in the earlier one (4 41)

Infection

Hellstrom (26) Carrol & Brennan (11) Rosenow (44) and Chute & Suby (12) considered that microorganisms especially ureasplitting bacteria were of great significance in the etiology of urolithiasis McGeown & Bull (37) on the other hand stated that the presence of urinary infection could not be demonstrated in many cases of renal calculi

In the present series urine culture was sterile in 70% of the cases The infection positivity tended to be more frequent with type C stone which had caused severe grades of ulceration of the mucosa and thus had created a good soil for the infection to get implanted Apparently in these as well as in other cases of

they are practically non existent in primary bladder stone disease of childhood

Oxalates

Hodgkinson (28) reported hyperoxaluria in a small percentage of cases of renal lithiasis De Albuquerque & Tuma (15) reported hyperoxal uria in women with recurrent or bilateral renal lithiasis On the other hand Gershoff & Prien (21) failed to find hyperoxaluria in their cases of recurrent renal lithiasis Xanthurenic acid excretion on the other hand was significantly greater in their patients than in controls After tryptophan load 11 of their 18 patients ex creted more than 30 mg of xanthurenic acid in 24 hours, an amount which Vilter *et al* (52) consider abnormal This indicates that Vitamin B_6 deficiency may not always be asso ciated with hyperoxaluria

Gershoff *et al* (22) and Valyasevi *et al* (49) have reported hyperoxaluria in village boys as compared to city boys in the bladder stone belt of Thailand This indirectly points to the role of oxaluria in primary bladder stone disease Significant oxalate crystalluria or increased oxalic acid excretion in 24 hours samples of urine was not seen in the present studies Only one patient excreted 36 4 mg of xanthurenic acid after tryptophan load

Hydrogen ion concentration of urine

It is well known that calcium phosphate and magnesium ammonium phosphate precipitate at a pH of 7 0 or higher whereas calcium oxalate precipitates over the entire physiological pH range In the present series pH of urine was less than 7 0 in 87 2% of the bladder stone cases In the entire series crystalluria was negligible or absent It is obvious that too much emphasis cannot be attached to pH itself in the etiopathogeneais of bladder stone disease of childhood

Proteins

The cases of bladder stone disease of child hood show in general some evidence of growth retardation indicated by relatively lower weights and heights when compared with well fed children In 15 cases the plasma albumin levels were less than 3 5 g/100 ml The high globulin levels in these patients bring the total protein values to within normal limits or even higher The low albumin and high globulin lev els were observed in the control children as well The raised globulin levels in the patients and the controls is probably on account of activity of immune response in childhood

Aurora *et al* (4) reported raised levels of serum mucoproteins in a small number of cases of bladder stones in children In the present series the serum mucoproteins in bladder stone cases were higher than control children This rise is of significance in view of the fact that stone matrix is rich in mucoproteins and thus may be etiologically related

The important role of urinary mucoprotein in the formation of stone matrix has been high lighted by King & Boyce (33) and Keutel *et al* (31) Boyce *et al* reported 3 to 13 times greater total urinary colloids in all the 17 patients with renal calcigerous stones than in the 14 normal individuals Keutel (30) remarks that all the fractions of urinary mucoprotein (TNDS) are quite fluctuant in urine from stone formers and that the supranormal values can be found in pathological urine from non stone cases In the present series, the urinary mucoproteins (the Andersen Maclagan material) lev els were raised in the bladder stone cases as compared to controls Wherever values of seromucoids as well as of uromucoids were avail able there was no definite correlation This was to be expected as the site of uromucoids (Tamm & Horsfall mucoproteins) formation has been shown to be kidney rather than the blood (31) It need be mentioned that the urinary muco proteins which contains many substances pri marily consist of the Tamm & Horsfall muco protein (Boyce W H—Personal Communication)

There is no correlation between the values of uromucoids and radiological changes ob served after intravenous pyelography in blad der stone cases of present series At times the



Fig 5 Histological section of a stone to show the multiple round to polyhedral cellars having common walls Each cellar has a central area hung from its walls by thin threads of matrix H & E ×30

aggregate while matrix is an adventitious inclusion a protein adsorbate upon the crystal faces In the present study the histological examination of some of the stones suggests that the matrix is laid down first as multiple round to polyhedral cellars having common walls In some of the cellars the walls become thicker to form the fibrillar matrix (Figs 5 and 6) It is possible that the rate of breakdown of the walls of the cellars determines the type of stone In cases where the walls of the cellars persist for long time and increase in thickness the type C stones are formed whereas in instances where the walls break down early with alignment of the remnants of the walls into fibrillar matrix a type B stone results When the breakdown is very rapid the matrix is chiefly non fibrillar and a type A stone results The factors which determine the rate of break down of the walls of the cellars could be variations in the pH and ionic strength of the urinary constituents besides the physical forces acting during the growth of the stone

The morphological studies of bladder stone matrices have shown the abundance of PAS positive diastase resistant and Alcian blue negative neutral mucopolysaccharides with a variable mixture of acid mucopolysaccharides (4 41) Aurora & Gupta (3) studying nephrocalcinosis in rabbits have shown that in the

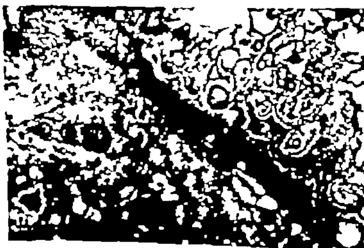


Fig 6 Another area of the stone depicted in Fig 5 to show how the fibrillar and amorphous matrices are laid down (Alcian Blue P A S) ×30

stone, infection could be the result rather than the cause

III Pathogenesis

It has been shown in the preceding paragraphs and highlighted in Table 7 that factors like hypercalciuria hyperoxaluria, hyperparathyroidism and infection, which are often held to be responsible for renal lithiasis cannot be incriminated in the problem of bladder stone disease of childhood nor can hypovitaminosis A be implicated From these observations it is obvious that bladder stone disease of childhood is an entity quite distinct from renal lithiasis

The questions that now need be answered are

(a) What is the site of stone formation in bladder stones of childhood? Does the stone descend from the kidneys to grow in the bladder or is it formed in bladder itself?
(b) What is the possible mechanism of formation of the stone?

(*a*) *Site of formation of bladder stones*

Andersen *et al* (1) have suggested that all bladder stones to begin with are formed in the kidneys This belief is based on their analysis of 30 stones It is suggested by them that the nucleus of bladder stone is formed in the kidney and further phosphatic deposits take place in the bladder If it be so the composition of the nuclei of bladder stones and those of kidney stones should be alike In their own analysis Andersen *et al* found considerable differences in the composition of bladder stone and kidney stone nuclei The data of Ranganathan quoted by Andersen *et al* further points to the marked difference in the composition of bladder and renal stones Moreover it would also be expected at least in some cases of bladder stones that kidneys also show stone formation The radiological studies of 61 cases of the present series including intravenous pyelography have failed to reveal even a single case where bladder stone disease was associated with kidney stone The finding of hydroureter and/or hydronephrosis in 68 7° of cases in which intravenous pyelography was done suggests a back pressure effect on these organs It was observed that the average duration of symptoms in cases without hydroureter was 5 2 months and in those showing hydroureter with or without hydronephrosis it was 11 27 months The average size of the stone in the two groups was 4 56 and 7 61 cm³ respectively Sanong Unakul (personal communication) from Thailand believes that kidney and bladder stones are independent of each other

The recurrence of renal lithiasis is well documented Surtherland (46) studied 345 cases of upper urinary tract lithiasis and recorded recurrence in 28 % within 2 years 58 4 % within 5 years and 87 6 % within 10 years This is in contrast to what is observed in bladder stone disease of childhood

Andersen *et al* (1) found only one case of recurrence without any obvious predisposing cause out of 103 patients Tiwari (47) followed 53 of the 100 cases of bladder stone in children for a period of 4 to 18 months without any evidence of recurrence Our epidemiological studies (5) also support the absence or rarity of recurrence of bladder stone in children after a surgical removal

The features mentioned above suggest that the bladder stones in childhood are formed in the bladder rather than in the kidney

(*b*) *Mechanism of formation of bladder stone*

The two chief components of stones are the matrix and the minerals The matrix is present either as fibrillar component or as an amorphous matrix The minerals are embedded in the amorphous matrix or aligned along the matrix fibrils The significance of this intimate relationship between the minerals and matrix is a controversial issue Boyce & co workers (6 8–10) extensively reported on the stone matrix and have highlighted the importance of the matrix which extracts the salts from the urine to form the stone On the contrary Fried & Vermeulen (20) and Vermeulen *et al* (51) state that stone formation is basically a crystalline



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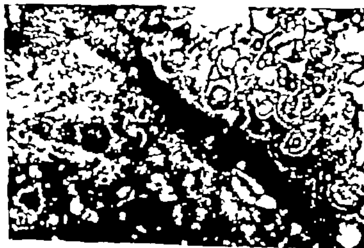


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9 — Crystal Matrix interrelations in calculi *J Urol 81* 351 1959
10 Boyce W H Pool C S Meschan I & King J S Jr Organic matrix of urinary calculi Microradiographic comparison of crystalline structure with microscopic and histochemical studies *Acta Radiol 50* 543 1958
11 Carroll G & Brennan R V The urea splitting organisms in the formation of urinary calculi *J International College of Surgeons 17* 809 1954
12 Chute R & Suby H I Prevalence and importance of urea splitting bacterial infections of the urinary tract in the formation of calculi *J Urol 44* 590 1940
13 Coelho G Morbidity among children *Indian J Child Health 4* 74 1955
14 Conway N S Maitland A I L & Rennie J B The urinary citrate excretion in patients with renal calculi *Brit J Urol 21* 30 1949
15 De Albuquerque P F & Tuma M Investigations on urolithiasis II Studies on oxalate *Trans Amer Ass Genitourin Surg 53* 196 1961
16 Dempsey F F Forbes A P Melick R A & Henneman P H Urinary oxalate excretion *Metabolism 9* 52 1960
17 Elliot, J S Vitamin A and Urolithiasis A review *Calif Med 80* 462 1954
18 Erickson W J & Feldman J B Signs of Vitamin A deficiency in the eye correlated with urinary lithiasis A report of clinical studies and investigations on twenty five patients *JAMA 109* 1706 1937
19 Flocks R H Calcium urolithiasis The role of calcium metabolism in the pathogenesis and treatment of calcium urolithiasis *J Urol 43* 214 1940
20 Fried F A & Vermeulen C W Artificial uric acid concretions and observations on uric acid solubility and supersaturation *Invest Urol 2* 131 1964
21 Gershoff S N & Prien E L Excretion of urinary metabolites in calcium oxalate urolithiasis Effect of tryptophan and Vitamin B administration *Amer J Clin Nutr 8* 812 1960
22 Gershoff S N Prien E L & Chandrapanond, A Urinary stones in Thailand *J Urol 90* 285 1963
23 Halstead S B Bladder stone in Thailand A review of the problem *Amer J Trop Med 10* 918 1961
24 Halstead S B & Valyasevi A Studies of bladder stone disease in Thailand III Epidemiological studies in Ubol Province *Amer J Clin Nutr 20* 1329 196
25 Harrison G A Chemical methods in clinical medicine J & A Churchill Ltd London 1957 pp 43 55
26 Hellstrom J Aetiological and therapeutic experiences concerning kidney and ureteric stones *Brit J Urol 21* 9 1949
27 Higgins C C Urinary lithiasis Experimental production and solution with clinical application and end results *J Urol 36* 168 1936
28 Hodgkinson A The urinary excretion of oxalic acid in nephrolithiasis *Proc Roy Soc Med 51* 970 1958
29 Hodgkinson A & Pyrah L N The urinary excretion of calcium and inorganic phosphate in 344 patients with calcium stone of renal origin *Brit J Surg 46* 10 1958
30 Keutel H J Fractionation of kidney stone matrix *Urol Int 19* 233 1965
31 Keutel H J King J S Jr & Boyce W H Further studies of uromucoids in normal and stone urine *Urol Int 17* 3 4 1964
32 King E J & Wootton I D P *Micro-analysis in medical biochemistry* J & A Churchill Ltd London 1959 3rd ed.
33 King J S Jr & Boyce W H Immunological studies on serum and urinary proteins in urolith matrix in man *Ann NY Acad Sci 104* (*Art 2*) 579 1963
34 Lowsley O S & Kirwin T J *Clinical urology* Williams & Wilkins Co Baltimore 1940 vol II pp 1025–1026
35 McCarrison R A lecture on the causation of stone in India *Brit Med J 1* 1009 1931
36 — The experimental production of stone in the bladder Part IV *Indian J Med Res 15* 801 1928
37 McGeown M G & Bull G M The pathogenesis of urinary calculus formation *Brit Med Bull 13* 33 1957
38 McIntosh H W & Seraglia M Urinary excretion of calcium and citrate in normal individuals and stone formers with variation in calcium intake *Canad Med Ass J 89* 1242 1963
39 Nabel J D & Riordan F H Hyperparathyroidism suggested by a primary vesical calculus *Amer J Surg 97* 117 1959
40 Passmore R Observations on the epidemiology of stone in the bladder in Thailand *Lancet 1* 638 1953
41 Ramalingaswami V & Aurora A L Nutritional aspects of calculus disease of the urinary tract *Fed Proc 20* (*No 1 Part III*) 317 1961
42 Rangachari V Ocular conditions associated with vitamin deficiencies *Antiseptic 54* 591 1957
43 Rao P T Customs of infant feeding in South India *Indian Child Health 6* 347 1957
44 Rosenow E C Jr Renal calculi A study of papillary calcification *J Urol 44* 19 1940
45 Steiner M Zuger B & Kramer B Production of renal calculi in guinea pigs by feeding them a diet deficient in Vitamin A *Arch Path 27* 104 1939
46 Sutherland J W Recurrence following operations for upper urinary tract stone *Brit J Urol 26* 22 1954
47 Thwin C P Bladder stones in children *Indian Practitioner 11* 1055 1958

early stages there was a gradual thickening of basement membrane of the tubules affected by calcification, due to the deposition of PAS positive material At a later stage a small amount of Alcian blue positive material was visible *in most severely affected tubules It is* possible that a part of the PAS positive material gets converted into Alcian blue positive mucopolysaccharides In the stone matrices under study a similar change could explain the variable amounts of acid mucopolysaccharides This is not to suggest that all such material is derived in this fashion and none comes from the urine

The morphological studies of the bladder biopsies have shown the abundance of PAS positive material in the dark staining cells which predominate in the bladder urothelium of cases of vesical calculi

It is believed that a wave of change from clear cells to dark cells and back to clear cells is *normally present At times of stress* this wave is disturbed so that the dark granular cells do not revert to clear cells till the stress is over The calculogenic factors might be acting in a similar fashion increasing the secretion of PAS positive material and thus favouring initiation of stone What factors or attributes *of micro-environment bring about these* cellular changes in the urothelium need further study It appears that neutral mucopolysaccharides elaborated by the dark granular cells under the influence of the calculogenic factors form a nidus which remains sticking to the mucosal surface till crystalline deposits give it an irregular shape and the stone starts growing

It is not improbable that there are certain inhibitory substance(s) in the urine of children, the derangement of which by a transiently acting factor brings *about the initiation of stone*

SUMMARY

A clinico pathological study of 61 cases of bladder stone in children was undertaken The patients showed some evidence of growth failure In none of the patients renal or ureteral stone could be visualized

The serum calcium and plasma phosphorus levels were within normal limits A significant finding in these cases was raised serum mucoprotein levels The urinary mucoprotein levels in patients were one and a half to two times higher than in control children Hypoalbuminemia was observed in 36 6% of the cases

Morphological studies of bladder biopsies revealed dark granular cells rich in PAS positive material

From the observations made it has been suggested that the bladder stone disease of *childhood is an entity distinct from* renal lithiasis The possible factors in the etiopathogenesis of the disease have been discussed

ACKNOWLEDGEMENTS

Mrss Roche Limited and Mrss Glaxo Limited very kindly supplied Vitamin A standard

Thanks are due to the *Director Principal* Maulana Azad Medical College and Associated Irwin and G B Pant Hospitals for permission to publish this work

REFERENCES

1 Andersen D A Sriramachari S & Khandwale M K Investigations into relationship between *bladder stones and malnutrition Indian J Med Sci 17* 617 1963
2 Anderson A J & Maclagan N F The isolation and estimation of urinary mucoproteins *Biochem J 59* 638 1955
3 Aurora A L & Gupta D N Nephrocalcinosis in rabbit *Indian J Med Sci 21* 250 1967
4 Aurora A L Ramalingaswami V & Gaitonde P D Bladder stone disease in children in Delhi area *J Urol 91* 347 1964
5 Aurora A L Taneja O P & Gupta *D N* Bladder stone disease of childhood I An epidemiological study *Acta Paediat Scand 59* xxx 1970
6 Boyce W H & Garvey F K The amount and nature of the organic matrix in urinary calculi a review *J Urol 76* 213 1956
7 Boyce W H Garvey F K & Goven C E Abnormalities of calcium metabolism in patients with idiopathic urinary calculi Effect of oral administration of sodium phytate *JAMA 166* 1577 1958
8 Boyce W H & King J S Jr Present concepts concerning the origin of matrix and stones *Ann NY Acad Sci 104 (Art 2)* 563 1963

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SERUM CREATININE VALUES IN HEALTHY CHILDREN

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Serum creatinine values were determined in a group of 408 children simultaneous with a study of the normal values of serum immunoglobulins (5)

The results appear to be of value in the assessment of serum creatinine levels in patients

MATERIAL AND METHODS

The study involved 408 healthy children (200 boys and 208 girls) aged 4 13 with an equal distribution over the various age groups

Fasting blood samples were obtained by venipuncture at school The samples were allowed to clot in glass tubes at room temperature Within two hours the serum was separated by centrifugation and frozen at − 20°C

The true creatinine concentration was determined according to De Vries & Daemelaar (6) The volume of the sample was scaled down from 1 ml to 0 05 ml as suggested by Renooij van Haga (unpublished) using polyethylene pipettes of the Sanz type (4)

The procedure was as follows

50 μl serum was mixed with 50 μl Fuller's earth suspension (30 mg Fuller's earth in 1 ml 0 36 N HCl) in a microtube After thorough mixing it was allowed to stand for 5 10 min 350 μl distilled water was added After shaking the suspension was centrifuged at 8000 g for 3 min whereupon the supernatant was decanted The sediment was washed with 400 μl 0 01 N HCl which was discarded after centrifugation

100 μl 0 05 M MgCl solution was added to the sediment and mixed 400 μl Picrate reagent was added (0 3 g 100 ml picric acid in 0 2 N NaOH) and mixed repeatedly over 20 min the mixture was then centrifuged at 8000 g for 3 min The optical density was determined at 514 nm in a microcuvette (optical path length 10 mm volume 120 μl) Calibration was carried out in duplicate with two standard solutions containing 1 and 2 mg creatinine per 100 ml A blank was determined in an identical manner using water instead of the serum specimen

The results obtained with this method represent the true creatinine values as creatinine free of chromogens is absorbed on Fuller's earth The scaling down of the sample volume does not affect the results The precision of the analytical procedure was calculated from (a) duplicate analyses ($n = 34$) and (b) the day-by-day variations ($n = 29$) of a reference serum (actual concentration 2 5 mg creatinine per 100 ml) Calculation of standard deviations yielded an identical value of ±5% for both methods

RESULTS

The different values of the serum creatinine concentrations were plotted against age and body surface area Body surface areas were calculated according to Dubois & Dubois

We found a wide scattering of the individual creatinine values in relation to age and body surface for both boys and girls (mean values standard deviation range see Table 1) However calculation of the mean values of the creatinine concentrations in relation to age and body surface disclosed a linear relationship (Figs 1 and 2) For the calculation of regression see statistical appendix

DISCUSSION

We found a considerable variation in the creatinine values This cannot be explained by the influence of exercise on serum creatinine J B Henry found that even intensive exercise over

48 Unakul S Urinary stones in Thailand A statistical survey *Sinraj Hospital Ga ette 13* 199 1961
49 Valyasevi A Halstead S B & Dhanmitta S Studies of bladder stone disease in Thailand VI Urinary studies in children 2–10 years old resident in a Hypo and hyperendemic area *Amer J Clin Nutr 20* 1362 1967
50 Varley H *Practical clinical biochemistry* William Heinemann Medical Books Ltd London 1958 2nd ed
51 Vermeulen C W Lyon E S & Fried F A On the nature of the stoneforming process *J Urol 94* 176 1965
52 Vilter R W Mueller J F Glazer H S Jarrold T Abraham J Thompson C & Hawkins V R The effect of Vitamin B deficiency induced by desoxypyridoxine in human beings *J Lab Clin Med 42* 335 1953
53 Wachstein M & Gudaitis A Detection of Vitamin B_6 deficiency Utilization of an improved method for rapid determination of xanthurenic acid in Urine *Amer J Clin Path 22* 652 1952
54 Weimer H E & Moshin J R Serum glycoprotein concentrations in experimental tuberculosis of guinea pigs *Amer Rev Tuberculosis 67* 594 1953

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Key words Bladder stones children seromucoids uromucoids hypercalciuria hyperoxaluria bladder biopsy dark and clear cells periodic acid Schiff (PAS) positive material fibrillar matrix amorphous matrix

	9	10	11	12
[illegible] 486 091 32–0.64)	21 0.487 0.101 (0.31–0.62)	23 0.549 0.083 (0.37–0.66)	33 0.560 0.086 (0.42–0.78)	15 0.598 0.112 (0.41–0.77)
[illegible] 479 076 34–0.60)	20 0.497 0.085 (0.34–0.69)	25 0.500 0.091 (0.31–0.67)	32 0.562 0.096 (0.36–0.84)	15 0.587 0.084 (0.49–0.80)
9,200 10 073	10 075 10 950	10 950 11 825	11 825 1 700	>12 700
27 0.504 0.092 (0.33–0.69)	28 0.525 0.105 (0.35–0.70)	18 0.542 0.096 (0.31–0.67)	15 0.572 0.068 (0.48–0.75)	23 0.570 0.103 (0.39–0.78)
31 0.506 0.098 (0.32–0.69)	25 0.481 0.113 (0.31–0.84)	21 0.545 0.059 (0.43–0.64)	18 0.507 0.087 (0.35–0.67)	22 0.592 0.078 (0.47–0.80)

(see statistical appendix) However we might have found differences between the sexes if we had been able to compare the values on the basis of lean body mass

We publish these results because we have the impression that the graphs in Figs 1 and 2 may have practical significance in the follow up and assessment of creatinine values in individual patients Longitudinal studies will show whether this impression is correct

SUMMARY

This paper presents the normal serum creatinine values in 408 healthy children aged 4–13 All determinations were carried out with a minimum volume of 50 μl serum specimen Mean values standard deviations and regression lines were calculated in relation to age and body surface for practical reasons of follow up and assessment in individual patients

Statistical appendix

Children were classified according to age body surface area and sex creatinine levels were studied in relation to this classification (Table 1)

It is clear that the influence of sex is small and not significant Regression lines were therefore calculated for boys and girls together (Table 2)

The two regression lines from Figs 1 and can be expressed as follows

Table 2 *Analysis of regression*

Source of variation	d f	Sum of squares	Mean square	F	P
Total	407	4.522	0.011		
Regression on age	1	1.606	1.606	223.662	0.0000
Error	406	2.915	0.007		
Regression on body surface area	1	1.443	1.443	190.223	0.0000
Error	406	3.079	0.008		
Regression on both	2	1.636	0.818	114.758	0.0000
Error	405	2.886	0.007		

Table 1 *Serum creatinine levels with standard deviations in relationship to age and body surface area*

Age (years)	4	5	6	7
Boys (number)	10	21	27	26
Mean creatinine value	0 362	0 415	0 429	0 45[illegible]
Standard deviation	0 066	0 076	0 072	0 08[illegible]
Range	(0 23–0 46)	(0 29–0 63)	(0 32–0 60)	(0 23–0 59)
Girls (number)	16	22	22	34
Mean creatinine value	0 382	0 400	0 432	0 439
Standard deviation	0 058	0 065	0 083	0 097
Range	(0 30–0 48)	(0 28–0 57)	(0 29–0 56)	(0 27–[illegible])

	Body surface area in square centimetres			
	<6 575	6 575 7 450	7 450 8 325	8 325 9 200
Boys (number)	6	20	31	[illegible]
Mean creatinine value	0 430	0 389	0 417	0 433
Standard deviation	0 129	0 053	0 082	0 078
Range	(0 23–0 63)	(0 23–0 49)	(0 29–0 60)	(0 2[illegible]–0 59)
Girls (number)	15	22	20	34
Mean creatinine value	0 390	0 384	0 423	0 443
Standard deviation	0 053	0 080	0 077	0 078
Range	(0 30 0 48)	(0 28–0 57)	(0 29–0 61)	(0 27–0 60)

extended periods did not influence serum creatinine (1) Our results are in agreement with those of Kuhlbach *et al* (3) and those of Josephson *et al* (2) who made similar studies of smaller numbers of healthy children

There was no significant difference between the creatinine values for boys and girls with regard to age as well as to body surface area

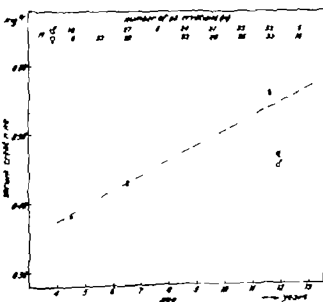


Fig 1 Mean values of serum creatinine in relation to age of 200 healthy boys and 208 healthy girls Correlation coefficient between creatinine and age = 0 596

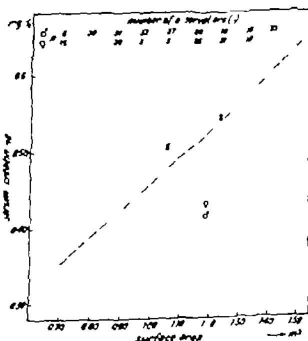


Fig 2 Mean values of serum creatinine in relation to body surface of 200 healthy boys and 208 healthy girls Correlation coefficient between creatinine and body surface = 0 565

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IMPAIRED LYMPHOCYTE TRANSFORMATION AND CHROMOSOMAL ABNORMALITIES IN FATAL GRANULOMATOUS DISEASE OF CHILDHOOD

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In recent years fatal granulomatous disease of childhood has been characterized as an inherited disorder that usually presents clinically in male infants with recurrent and persistent infection (3 5) and this has been associated with an abnormality of leucocyte function (8) Various tests have been described to record a defective intracellular bactericidal action despite normal phagocytosis (8 12) An intracellular nitro-blue tetrazolium (NBT) method first showed the asymptomatic intermediate carrier state in the mothers of such patients and this suggested a sex linked transmission for the disease (12) This clinical syndrome has now been described in several girls and the fact that the parents have normal leucocyte function suggests that the disease may also be transmitted as an autosomal recessive trait

More recently a further case of fatal granulomatous disease was described where an abnormal leucocyte bactericidal activity and nitro-blue tetrazolium (NBT) reduction was also detected in the father and upon this a new mode of genetic transmission was proposed (11) This case is again reported here firstly as it presented two new additional *interesting* features and also since the original abnormality detected in the father of this patient could not be confirmed

Case Record

D B a 5 year-old boy first presented at the age of 4 months with infection Since the first admission the patient has been seen on many occasions with recurrent and persistent infections The most common site of infection was the respiratory tract After the age of 14 months when hepatosplenomegaly was first recorded recurrent episodes of osteomyelitis and chronic granulomatous lymphadenitis became the most troublesome sites of persistent infection

The family history showed that both parents and two siblings a boy aged 8 and a female aged 6 were alive and well Another sibling a male had died previously with septicaemia at the age of 7 weeks

MATERIALS AND METHODS

In vitro lymphocyte transformation to phytohaemagglutinin (PHA) was performed on peripheral blood 5 ml of blood was initially taken into a sterile plastic syringe containing 0 5 ml of non ionic (phenol free) heparin 0 5 ml aliquots of this were then added to 7 0 ml of tissue culture media (6 ml of 10× bicarbonate buffered 199 solution with 1 ml of freshly inactivated AB plasma) Penicillin and streptomycin were added to achieve a final concentration of 100 international units (I U) and 74 5 I U /ml respectively Finally either 0 1 ml of PHA or normal physiological saline (as a control) were added to the cultures prior to incubation

On occasion the blood cells were first washed three times in an excess of buffered 199 solution prior to the addition of PHA Again in other cultures 0 5 ml of serum or serum protein fractions were added before the addition of PHA

All cultures were incubated for 72 hours at 37 C before colchicine (0 03 ml) was added After an hour's further incubation blood films were made from the centrifuged pellet of cells with the addition of two drops of bovine serum albumin These films were subsequently stained with May–Grünwald Giemsa and examined by conventional microscopy In each preparation 1000 nucleated cells were classified and the numbers of "blast forms" and cells in mitosis were recorded

creatinine in mg/100 ml = 0 254 + 0 026 × age in years
creatinine in mg/100 ml = 0 190 + 0 274 × body surface area in m

To conclude the correlation coefficients between the three factors are given

creatinine – age = 0 596
creatinine – body surface area = 0 565
age – body surface area = 0 895

REFERENCES

1 Henry J B Clinical chemistry *In* I Davidson & J B Henry (eds) *Clinical diagnosis by laboratory methods* W B Saunders New York 1969 14th edn p 545

2 Josephson B Fürst P & Jammark O Age variations in the concentration of non protein nitrogen creatinine and urea in blood of infants and children *Acta Paediat Scand* Suppl *135* 111 1962

3 Kuhlbach B Pasternack A Launiala K & Stenberg M Serum creatine and creatinine in children and adolescents *Scand J Clin Lab Invest* *22* 47 1968

4 Sanz M C Ultramicro methods and standardization of equipment *Clin Chem* *3* 406 (Suppl) 1957

5 Stoop J W Zegers B J M Sander P C & Ballieux R E Serum immunoglobulin levels in healthy children and adults *Clin Exp Immun* *4* 101 1969

6 de Vries L A & van Dreumel J J Bepaling van creatinine in serum volgens Folin *In* E Gorter & W C de Graaf (eds) *Klinische Diagnostiek* H E Stenfert Kroese N V Leiden 1955 7th edn p 278

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IMPAIRED LYMPHOCYTE TRANSFORMATION AND CHROMOSOMAL ABNORMALITIES IN FATAL GRANULOMATOUS DISEASE OF CHILDHOOD

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In recent years fatal granulomatous disease of childhood has been characterized as an inherited disorder that usually presents clinically in male infants with recurrent and persistent infection (3 5) and this has been associated with an abnormality of leucocyte function (8) Various tests have been described to record a defective intracellular bactericidal action despite normal phagocytosis (8 12) An intracellular nitro-blue tetrazolium (NBT) method first showed the asymptomatic intermediate carrier state in the mothers of such patients and this suggested a sex linked transmission for the disease (12) This clinical syndrome has now been described in several girls and the fact that the parents have normal leucocyte function suggests that the disease may also be transmitted as an autosomal recessive trait

More recently a further case of fatal granulomatous disease was described where an abnormal leucocyte bactericidal activity and nitro-blue tetrazolium (NBT) reduction was also detected in the father and upon this a new mode of genetic transmission was proposed (11) This case is again reported here firstly as it presented two new additional interesting features and also since the original abnormality detected in the father of this patient could not be confirmed

Case Record

D B a 5 year-old boy first presented at the age of 4 months with infection Since this first admission the patient has been seen on many occasions with recurrent and persistent infections The most common site of infection was the respiratory tract After the age of 14 months when hepatosplenomegaly was first recorded recurrent episodes of osteomyelitis and chronic granulomatous lymphadenitis became the most troublesome sites of persistent infection

The family history showed that both parents and two siblings a boy aged 8 and a female aged 6 were alive and well Another sibling a male had died previously with septicaemia at the age of 7 weeks

MATERIALS AND METHODS

In vitro lymphocyte transformation to phytohaemagglutinin (PHA) was performed on peripheral blood 5 ml of blood was initially taken into a sterile plastic syringe containing 0 5 ml of non toxic *(phenol free)* heparin 0 5 ml aliquots of this were then added to 7 0 ml of tissue culture media (6 ml of 10* bicarbonate buffered 199" solution with 1 ml of freshly inactivated AB plasma) Penicillin and streptomycin were added to achieve a final concentration of 100 international units (I U) and 74 5 I U /ml respectively Finally either 0 1 *ml of PHA* or normal physiological saline (as a control) were added to the cultures prior to incubation

On occasion the blood cells were first washed three times in an excess of buffered "199 solution prior to the addition of PHA Again in other cultures 0 5 ml of serum or serum protein fractions were added before the addition of PHA

All cultures were incubated for 72 hours at 37 *C* before colchicine (0 03 ml) was added After an hour s further incubation blood films were made from the centrifuged pellet of cells with the addition of two drops of bovine serum albumin These films were subsequently stained with May–Grünwald–Giemsa and examined by conventional microscopy In each preparation 1000 nucleated cells were classified and the numbers of blast forms and cells in mitosis were recorded

creatinine in mg/100 ml = 0 254 + 0 026 × age in years
creatinine in mg/100 ml = 0 190 + 0 274 × body surface area in m

To conclude the correlation coefficients between the three factors are given

creatinine	– age	= 0 596
creatinine	– body surface area	= 0 565
age	– body surface area	= 0 885

REFERENCES

1 Henry J B Clinical chemistry *In* I Davidson & J B Henry (eds) *Clinical diagnosis by laboratory methods* W B Saunders New York 1969 14th edn p 545
2 Josephson B Furst P & Jammark O Age variations in the concentration of non protein nitrogen creatinine and urea in blood of infants and children *Acta Paediat Scand* Suppl *135* 111 1962
3 Kuhlbach B Pasternack A Launiala K & Stenberg M Serum creatine and creatinine in children and adolescents *Scand J Clin Lab Invest* *22* 47 1968
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Fig 1 Serum inhibitor to *in vitro* lymphocyte transformation

no numerical chromosome abnormalities in the propositus although in 20 cells (5) structural abnormalities as of the kind shown in Fig 4 were observed These were not restricted to one particular chromosome or group of chromosomes

The abnormalities obtained on both occasions from the mother's cultures were in certain respects similar to those obtained in the propositus (as in Fig 4) Polyploidy and hyperdiploidy were found in a small proportion of her cells but structural abnormalities were obtained in 40 % of the cells examined on the prior occasion (Figs 5 and 6) the majority of which were diploid on both occasions The cells containing 45 chromosomes could have been formed from cell breakage during the preparation of the slides since different chromosomes were missing in each cell examined

The chromosomes of the propositus father were normal in all respects

DISCUSSION

This case of fatal granulomatous disease of childhood presented the characteristic clinical pattern of recurrent and persistent infection with hepatosplenomegaly and the grossly abnormal result with leucocyte phagocytosis and digestion test of Holmes *et al* (8) confirmed this diagnosis The fact that the patient's mother presents an intermediate response in this test would suggest that this patient is very similar to the previously described sex linked cases (6) In this respect the normal results obtained for the father would support this The limitation of the techniques used by Thompson and her co-workers (11) to demonstrate an apparent abnormality in the father on two different occasions have been stressed by others (13) (7) and this could account for the contrasting results There is an alternative explanation possible Alexander *et al* (1) noted a transient leucocyte dysfunction in several different disorders and it is conceivable that it is this which has been detected by Thompson *et al* (11) Meanwhile at this stage it seems unwise to modify any hypothesis concerning the genetic transmission of fatal granulomatous disease until either these contrasting results in

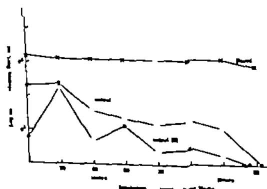


Fig Comparison of the destruction of *S au eus* by peripheral white cells obtained from two controls and the patient with fatal granulomatous disease

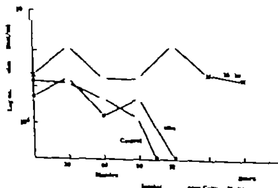


Fig 3 Comparison of the destruction of *S aureus* by peripheral white cells obtained from a control and the mother and father of a patient with fatal granulomatous disease

Table 1 *In vitro lymphocyte transformation to PHA*

Source of lymphocytes		of nucleated cells	
		Blast forms	Mitoses
Patient		4 6	2 2
Controls	1	57 0	3 0
	2	51 0	10 0
	3	50 2	1 2
	4	55 0	3 6
	5	45 6	1 6

Table 2 *The effect of washing the cells prior to stimulation with PHA*

	Treatment	of nucleated cells	
		Blast forms	Mitoses
Patient	Unwashed	15 6	0 8
	Washed and resuspended in normal control (1) sera	33 6	2 2
	Washed and resuspended in normal control (2) sera	47 0	4 2

Serum fractionation was initially performed with the addition of an equal volume of saturated ammonium sulphate solution The crude globulin precipitate resuspended in a small volume of normal saline was dialysed free of sulphate an aliquot retained (F) whilst the remainder was further fractionated through DEAE cellulose into three fractions (F F F) according to the method of Peterson & Sober (10) The original soluble serum fraction remaining after precipitation with the saturated ammonium sulphate was also dialysed free of sulphate and known as the crude albumin fraction (F_A) All fractions were finally dialysed against large volumes of normal physiological saline and sterilized by passage through a Millipore 0 25 filter prior to use Immunoelectrophoresis with goat anti human serum (Hyland Laboratories Los Angeles) was used to confirm the nature of the fractions

Polymorph phagocytosis test was performed according to the method of Holmes *et al* (8)

Cytogenetic examination was performed on 100 cytologically suitable cells obtained from peripheral blood as previously described by Brahim *et al* (4)

RESULTS

At 14 months of age when the child presented with recurrent infected ischiorectal fistulae and bilateral suppurative inguinal adenitis

In vitro lymphocyte transformation test in the patient showed a marked reduction in the number of "blast forms when compared with five normal controls (Table 1)

At 18 months of age when the patient presented with osteomyelitis of the right olecranon and right tibia

In vitro lymphocyte transformation was again impaired however a relatively high transformation index was obtained (% of blast cells) when the cells were washed (Table 2)

The transformation of normal lymphocytes from red cell compatible control samples was impaired on the addition of the patient's serum and this was later shown to be associated with the crude globulin F_G and F_1 serum fractions (Table 3) the latter was shown by immuno electrophoresis to be largely IgG (Fig 1)

At 4 years of age the infant was re admitted with recurrent and persistent infections and

(*a*) *The polymorph phagocytosis test* showed that the patient, compared with controls had normal phagocytosis but a diminished ability to digest bacteria (Fig 2) The mother was noted to have an intermediate level whilst the two other siblings and the father were normal in this test (Fig 3)

(*b*) *In vitro lymphocyte transformation* to PHA was normal (transformation index 73%)

(*c*) *Chromosome studies* on peripheral blood from the patient his mother (two observations) and father are shown in Table 4 There were

Table 3 *The inhibition of normal lymphocyte transformation with serum fractions from the patient*

Source of lymphocytes and serum fractions used		of nucleated cells	
		Blast forms	Mitoses
Normal (control) peripheral blood (washed)	Patient's whole serum	13 6	0 8
	Patient's F_A	34 6	1 0
	Patient's F_G	12 6	0 8
	Patient's F	10 8	0 4
	Patient's F	23 8	1 0
	Patient's F	23 0	1 2



Fig 1 Serum inhibitor to *in vitro* lymphocyte transformation

no numerical chromosome abnormalities in the propositus although in 20 cells (5) structural abnormalities as of the kind shown in Fig 4 were observed These were not restricted to one particular chromosome or group of chromosomes

The abnormalities obtained on both occasions from the mother s cultures were in certain respects similar to those obtained in the propositus (as in Fig 4) Polyploidy and hyperdiploidy were found in a small proportion of her cells but structural abnormalities were obtained in 40 % of the cells examined on the prior occasion (Figs 5 and 6) the majority of which were diploid on both occasions The cells containing 45 chromosomes could have been formed from cell breakage during the preparation of the slides since different chromosomes were missing in each cell examined

The chromosomes of the propositus father were normal in all respects

DISCUSSION

This case of fatal granulomatous disease of childhood presented the characteristic clinical pattern of recurrent and persistent infection with hepatosplenomegaly and the grossly abnormal result with leucocyte phagocytosis and digestion test of Holmes *et al* (8) confirmed this diagnosis The fact that the patient s mother presents an "intermediate response in this test would suggest that this patient is very similar to the previously described sex linked cases (6) In this respect the normal results obtained for the father would support this The limitation of the techniques used by Thompson and her co-workers (11) to demonstrate an apparent abnormality in the father on two different occasions have been stressed by others (13) (7) and this could account for the contrasting results There is an alternative explanation possible Alexander *et al* (1) noted a transient leucocyte dysfunction in several different disorders and it is conceivable that it is this which has been detected by Thompson *et al* (11) Meanwhile at this stage it seems unwise to modify any hypothesis concerning the genetic transmission of fatal granulomatous disease until either these contrasting results in

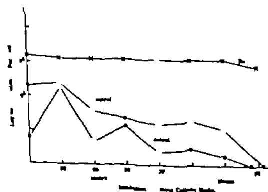


Fig 2 Comparison of the destruction of *S aureus* by peripheral white cells obtained from two controls and the patient with fatal granulomatous disease

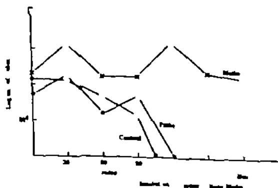


Fig 3 Comparison of the destruction of *S aureus* by peripheral white cells obtained from a control and the mother and father of a patient with fatal granulomatous disease

Table 4 *Chromosomal pattern in the peripheral blood of the parents and the patient with fatal granulomatous disease*

Subject	No of cells karyotyped	Chromosome counts						Total cells	Comment
		<44	45	46	47	>48	Poly		
Propositus	10	1	0	97	—	—	2	100	5 Abnormal Cells
Mother (1)	20	—	5	89	—	2	4	100	40 Abnormal Cells
(2)	5	1	3	91	—	—	5	100	20 Abnormal Cells
Father	3	1	1	97	—	—	1	100	Normal

the father of this patient are explained or other similar cases described

The impaired lymphocyte transformation to PHA originally noted was very surprising particularly when traced to the presence of a serum factor The transient nature of this was suggested when the *in vitro* response became normal when tested at 4 years of age The significance of this remains unknown however it is important to note that serum inhibitors to *in vitro* lymphocyte transformation exist in this and other conditions (9) and that an apparent impaired transformation does not necessarily primarily implicate the lymphocyte Good *et al* (6) in their review of cases with fatal granulomatous disease noted lymphocyte transformation to be normal and the case here is the first to be recorded where transformation was impaired

This case is also the first description of abnormal chromosomes in fatal granulomatous disease of childhood The significance of the abnormality and the incidence of such changes in this disease however, remain speculative Chromosomal abnormalities are essentially congenital or acquired and until further cases are studied the cause of the abnormalities here must be considered unknown If the chromosomal abnormalities in both the propositus and his mother are congenital this might be considered, in the absence of any abnormal cytogenetic findings in the father that the mode of transmission of this might be sex linked This in turn might be indirect evidence to favour a similar sex linked transmission of fatal granulomatous disease of childhood in this child Of course the chromosomal abnormalities in both the mother and the affected offspring may have

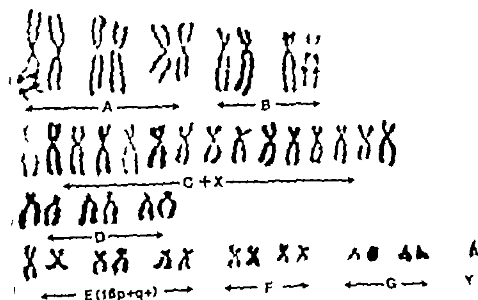


Fig 4 Karyotype of a cell from propositus showing enlargement of both arms of no 16 pair of chromosomes (not a constant feature in all cells) Two of the cells karyotyped from the mother also showed this feature

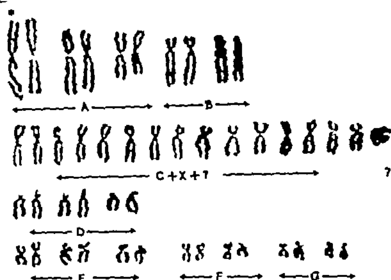


Fig 5 Karyotype of a cell from mother showing chromatid break (asterisk) and enlargement of long arm of a D chromosome

b en acquired independently and this view is probably favoured by the fact that the abnormalities were different If these chromosome abnormalities had b en acquired it would be interesting to know the cause The patient at the time of investigation was suffering from inevitable infection but the mother on both occasions when tested was apparently clinically normal and no infection was obvious It is conceivable that chromosomal abnormalities are a feature of both fatal granulomatous disease and its carrier state and if so might be a valuable diagnostic adjunct if further studies can confirm this



Fig 6 karyotype of another cell from mother showing chromatid break (asterisk) and ill-defined chromosome

Table 4 *Chromosomal pattern in the peripheral blood of the parents and the patient with fatal granulomatous disease*

Subject	No of cells karyotyped	Chromosome counts <44	45	46	47	>48	Poly	Total cells	Comment
Propositus	10	1	0	97	—	—	2	100	5 Abnormal Cells
Mother (1)	20	—	5	89	—	2	4	100	40 Abnormal Cells
(2)	5	1	3	91	—	—	5	100	20 Abnormal Cells
Father	3	1	1	97	—	—	1	100	Normal

the father of this patient are explained or other similar cases described

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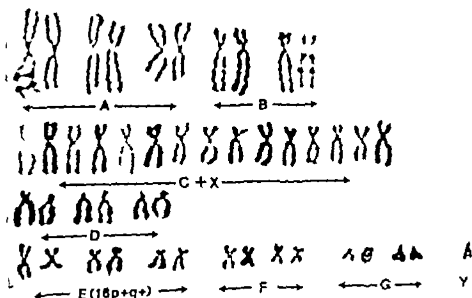


Fig 4 Karyotype of a cell from propositus showing enlargement of both arms of no 16 pair of chromosomes (not a constant feature in all cells) Two of the cells karyotyped from the mother also showed this feature

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THE USE OF AZATHIOPRINE IN REFRACTORY IDIOPATHIC THROMBOCYTOPENIC PURPURA IN CHILDREN

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Corticosteroids and splenectomy are accepted therapeutic measures for treatment of Idiopathic Thrombocytopenic Purpura (ITP) Bouroncle & Doan (3) reported 7 7 of 271 patients refractory to both modes of therapy and that 4 4 died from fatal bleeding These methods of treatment are not ideal since prolonged steroid therapy has undesirable and occasionally dangerous complications and splenectomy in children might be associated with life threatening and occasionally fatal infections (12) A logical extension of the concept that ITP is an autoimmune disorder (2) is to attempt to treat it by drugs capable of suppressing the immune response (1) Since azathioprine (Imuran) is an effective immunosuppressive agent is easily controlled and the least toxic of the immunosuppressive drugs its effectiveness in refractory ITP in children merits trial Preliminary results of the use of this drug predominantly in adults with ITP have been very encouraging (3 4 6 13) The purpose of this paper is to report our findings in 5 children with refractory ITP treated with azathioprine

MATERIALS AND METHODS

Five children (males and 3 females) with chronic ITP aged from 2 years to 1 years were evaluated for the results of therapy with azathioprine Of the patients 2 had had splenectomy and required steroids (1 2 mg/kg) post splenectomy for 3 to 36 months respectively for control of bleeding and 3 non splenectomized children required persistent steroid therapy to control symptoms

The following criteria were used for selection of patients to receive azathioprine therapy

1 A diagnosis of idiopathic thrombocytopenic purpura with bone marrow findings consistent with this diagnosis. Patient with secondary thrombocytopenic purpura were not included

2 The patient had been treated with steroids in a dose of 2 0 mg/kg/day for 4 6 weeks on at least two occasions and had relapsed requiring either maintenance steroid therapy to control bleeding with a platelet count below 40 000/mm or had relapsed post-splenectomy and required steroid therapy to control symptoms with platelets between 10–40 000/mm

Before azathioprine therapy was started the patients were closely supervised without steroid therapy for at least 6 weeks to obviate the possible influence of prolonged corticosteroid therapy and remission of thrombocytopenia with its cessation (5) The following base line tests were carried out liver function studies (total serum protein, albumin globulin alkaline phosphatase bilirubin and SGOT) Coombs test lupus preparations and test for platelet antibodies After this observation period azathioprine in a dose of 2 0 mg/kg day was given If there was no platelet response within 4 weeks the dose was increased to 3–4 mg/kg/day and if there was no response in 8 weeks a small dose of steroids (0 2 mg/kg/day) was added Complete blood count was carried out twice weekly for 5 weeks and thereafter every 2–3 weeks depending on the degree of leukopenia and platelet count

We were prepared to permit the leukocyte count to drop to 2000/mm and the hemoglobin level to drop 2 g per 100 ml before decreasing the dosage of the drug However this degree of hematologic depression

This work was supported by the Children's Blood Foundation Inc Azathioprine (Imuran) Burroughs Wellcome & Co (U.S.A.) Inc

SUMMARY

A previously described case of fatal granulomatous disease of childhood is presented here as certain previous findings could not be confirmed and since two unusual features were noted Upon the basis of impaired leucocyte function in the father of this patient a new mode of genetic transmission for the disease was proposed meanwhile we have been unable to confirm these original findings (11) This case in itself was interesting since firstly an impaired *in vitro* lymphocyte transformation was noted and this later related to the presence of a serum inhibitory factor Secondly chromosomal abnormalities were recorded in peripheral blood cells of both patient and his mother and this might be considered evidence to favour a sex linked transmission of the disease in this case

ACKNOWLEDGEMENTS

The authors wish to acknowledge the technical assistance of Mrs J Thomas

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REFERENCES

1 Alexander J W Windhorst D B & Good R A Improved tests for the evaluation of neutrophil function in human disease *J Lab clin Med 72* 136 1968
2 *Baehner R L* & Nathan D G Quantitative nitrobluetetrazolium test in chronic granulomatous disease *New Eng J Med 278* 971 1968
3 Berendes H Bridges R A & Good R A A fatal granulomatosis of childhood the clinical study of a new syndrome *Minn Med 40* 309 *1957*
4 Bishun N P Morton W R M & McLaverty B Macromethod for culturing leucocytes for chromosomal analysis *Lancet II* 315 1964
5 Bridges R A Berendes H & Good R A A fatal granulomatous disease of childhood the clinical pathological and laboratory features of a new syndrome *Amer J Dis Child 97* 387 1959
6 Good R A Quie P G Windhorst D B Page A R Rodey G E White J Wolfson J J & Holmes B H Fatal (chronic) granulomatous disease of childhood A hereditary defect of leukocyte function *Seminars in Hematology* v 215 1968
7 Gruch O C & Mauer A M Neutrophil Function and N B T Dye Reduction *Lancet II* 383 1969
8 Holmes B Quie P G Windhorst D B Pollara B & Good R A Fatal granulomatous disease of childhood An abnormality of phagocytic function *Lancet I* 1227 1966
9 Levene G M Turk J L Wright D J M & Grimble A G S Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis *Lancet II* 246 1969
10 Peterson E A & Sober H A *in* S P Colowick & N O Kaplan (eds) *Methods in Enzymology* Academic Press New York 1962 vol 2 p 3
11 Thompson E N Chandra R K Cope W A & Soothill J F Leucocyte abnormality in both parents of a patient with chronic granulomatous disease *Lancet I 799 1969*
12 Windhorst D B Holmes B & Good R A A newly defined X linked trait in man with demonstration of the Lyon effect in carrier females *Lancet I* 737 1967
13 Windhorst D B Inheritance of fatal granulomatous disease *Lancet II* 543 1969

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Key words Chronic granulomatous disease of childhood chromosomal abnormalities lymphocyte transformation inhibition

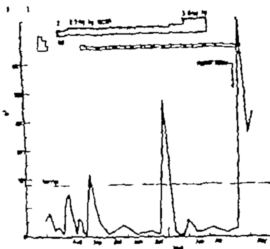


Fig 3 Course of disease in case 3 (S D B D 1/62)

... nisone 1 mg/kg/day for four months with good response followed by four months without therapy when she was well. Petechiae returned and she was given Prednisone 2 mg/kg/day with a good response. After 6 weeks steroids were tapered, petechiae and bruising returned. She was maintained without steroid therapy for 4 weeks. Azathioprine 2.5 mg/kg/day was given for six weeks with poor response. Steroids at 1 mg/kg/day were added to the regimen without sustained response. Azathioprine was increased to 5.0 mg/kg/day for an additional month when a raised SGOT was observed and the drug was discontinued. Since there had been no response for an 8 month period this patient was considered a failure to azathioprine therapy and splenectomy was performed with excellent results (Fig 3)

Case 4 (E. V – NYH no 106-46 80)

A 27 month-old girl developed purpura. The bone marrow findings were consistent with idiopathic thrombocytopenic purpura. She was treated with Prednisone 0.5 mg/kg/day for 6 months without response, then taken off all therapy for 7 months. Because of bruising and epistaxis a splenectomy was performed 13 months after the onset of the disease with good platelet response to 500 000/mm. She was well for 15 months post splenectomy when purpura and epistaxis reappeared. Steroid therapy 2 mg/kg/day was given for 12 weeks and tapered without rise of platelets over 30 000/mm³. After 10 weeks off therapy azathioprine 2.5 mg/kg/day was given for 3 months without response. Prednisone was added at 0.2 mg/day. She responded slowly over 8 months. Steroids were tapered over 4 months and azathioprine over an additional 8 month period. Platelets fell with upper respiratory infections but returned to normal thereafter and remained so eight months after therapy was stopped. The results of 2 years of therapy are excellent (Fig 4)

Case 5 (R. K. – NYH no 78 54-27)

In 1956 this boy developed easy bruising and severe epistaxis at 2 ½ years of age. A bone marrow examination was compatible with ITP. Platelet count was less than 10 000/mm. He was treated with Prednisone 1 mg/kg/day for three years with no improvement. The dose was increased to 2 mg/kg/day over the next year with some improvement but relapse with bruising and petechiae occurred when therapy was decreased and discontinued. A third course of steroids 2 mg/kg/day produced a rise in platelet count to 400 000/mm with subsequent fall when therapy was discontinued. Splenectomy was performed in May 1961 with good results. Platelets rose to 410 000/mm postsplenectomy. The patient had a relapse 2 ½ years later with petechiae and gastro-intestinal bleeding, platelet count was 34 000/

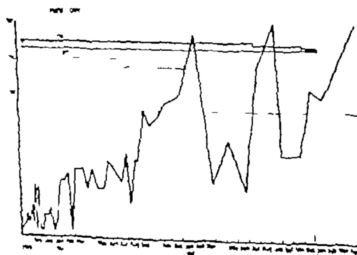


Fig 4 Course of disease in case 4 (E. V. B D 10 61 Splenectomy 2/55)

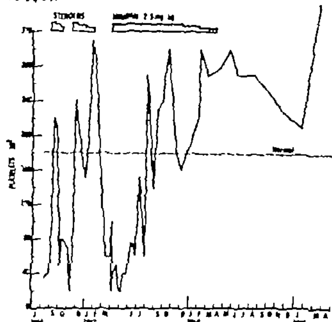


Fig 1 Course of disease in case 1 (P B B D 8/54)

was not observed Therapy was continued for 8 months before considered a failure and in successful cases was continued for at least 2 months after a satisfactory platelet count was obtained before the drugs were tapered Immunophoretic studies were done from time to time during therapy

CASE REPORTS

Case 1 (P B - NYH no 106 22 93)

A 12 year old boy had sudden onset of petechiae and bruising The bone marrow findings were consistent with ITP He was treated with Prednisone 2 mg/kg/ day for six weeks with rise of platelets to 220 000/ mm³ but relapsed with petechiae and ecchymosis and platelets fell to 20 000/mm³ when therapy was discontinued A second course of steroids in the same dose given for 10 weeks was followed by a rise of platelets to 310 100/mm but when the steroids were discontinued relapse occurred again Eight months after onset of symptoms azathioprine 2 5 mg/kg/ day was begun He had a slow response over a 4 month period with platelet count rising to 270 000 mm He was maintained for additional 4 months on the same dosage and then tapered slowly over 4 months Total duration of azathioprine therapy was 12 months The result of therapy was excellent (Fig 1)

Case 2 (C C - NYH no 107 86 99)

A 10 ¹/₂-year old girl had gradual onset of petechiae and ecchymosis The bone marrow aspiration was consistent with ITP She was placed on Prednisone at 1 mg/kg/day with good results Platelet count remained high at 220 000/mm as steroids were withdrawn Two weeks after discontinuing steroids the patient developed mumps with a return of ecchymosis and petechiae in 1 week with platelet count of 10 000/mm Steroids in the dose of 2 mg/kg/day again produced a good response and platelet count rose to 280 000/mm She was well for seven months without any treatment when symptoms returned and azathioprine was begun at 2 0 mg/kg/day She had a slow response over a 7 month period possibly accelerated by a rise in dosage to 3 0 mg/kg Total duration of therapy on azathioprine was 12 months She has remained well and asymptomatic 7 months after azathioprine was stopped The results are excellent (Fig 2)

Case 3 9 (S D NYH no 109 20 29)

A 4 /₂-year old girl had a sudden onset of petechiae and severe ecchymosis and the bone marrow findings were consistent with ITP She was treated with Pred

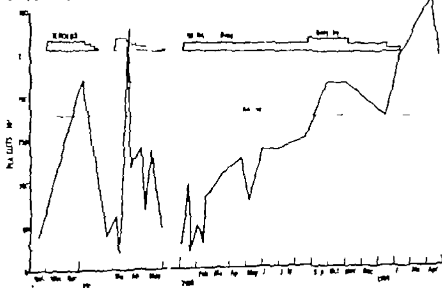


Fig 2 Course of disease in case 2 (C C B D 3/56)

Table 2 Toxicity observed during azathioprine therapy

Case no	Hemoglobin levels g/100 ml			Leukocyte levels /mm³			Liver function tests		
	Initial	Lowest	Present	Initial	Lowest	Present	Initial	During therapy	Present
1 P B	13 8	11 4	13 1	8 950	2 900	5 000	Normal	No change	Normal
2 C C	12 4	11 4	13 0	7 850	3 100	8 200	Normal	No change	Normal
3 S D	11 7	10 3	11 4	10 700	7 700	8 450	Normal	SGOT 55 (nl 20–40 U/ml)	Normal
								SGPT 11 9 (nl 5–40 U/ml)	Normal Normal
4 E V	1 5	10 6	11 7	8,900	3 150	6 600	Normal	No change	
5 R K	14 8	13 0	14 5	6 100	3 100	10 600	Normal	No change	Normal

mission without relapse after discontinuation of all therapy 2) Good excellent initial response 3) Relapsed requiring treatment or 4) Failure no response

An excellent result was obtained in 3 patients a good result in 1 and 1 patient failed to respond Of the patients who had responded 2 had not undergone splenectomy and 2 had relapsed post splenectomy The 2 post splenectomy patients required low dosage steroids for a temporary period in addition to azathioprine Th one pre splenectomy patient who failed to respond to azathioprine responded well to splenectomy

Table 2 summarizes toxicity observed during therapy Slight fall in hemoglobin was observed leukopenia occurred in 4 patients and minimal changes in SGOT occurred in only 1 patient All these changes were transient

Platelet antibodies were not found in any of the patients before therapy One patient had a transiently positive Coombs test which reverted to normal before azathioprine therapy

Immunophoretic studies done at the end of azathioprine therapy in cases 2 4 and 5 revealed normal levels of IgG IgG was 1480 mg/100 ml in case 2 1300 mg/100 ml in cases 4 and 5 with a normal range of 776–1647 mg/100 ml for the age IgA was at the upper limit of normal with values of 250 250 and 260 m /100 ml respectively for cases 2 4 and 5 with a normal range of 64–290 mg/100 ml IgM was normal in cases 2 and 4 with levels of 102 mg/100 ml and 106 ml/100 ml and slightly low in case 5 51 mg/100 ml with 60–140 mg/100 ml the range of normal After six weeks off therapy this level rose to 70 mg/100 ml

DISCUSSION

ITP in childhood is not necessarily a self limited disease of short duration Many cases become chronic and relapses are known to occur Although the etiology remains obscure many authors believe it to be an autoimmune phenomenon with spontaneous remission unlikely in those who become chronic

Since conventional therapy with corticosteroids and splenectomy have significant risks an alternate mode of therapy has been sought. A great deal of interest has been aroused in the suppression of autoimmune disease by antimetabolites Schwartz & Dameshek (11) first showed that mercaptopurine and thioguanine were useful in the treatment of autoimmune hemolytic anemia Since then these antimetabolites have been successfully used to treat other suspected immune diseases such as systemic lupus nephrotic syndrome rheumatoid arthritis and ulcerative colitis (6 8 15) In addition they have been used to suppress antibody formation at the time of tissue transplantation

More recently azathioprine has been used with increasing frequency because it has less toxicity less deleterious effect on the bone marrow and greater immunosuppressive effect although it is metabolized to its analogue mercaptopurine in the body (10) It has also been

Fig 5 Course of disease in case 5 (R K B D 12/53 Splenectomy 5/61)

mm³ A Coombs test was weakly positive at this time but became negative within 3 months and has remained negative Fresh frozen plasma 30 mg/kg did not produce a platelet rise Platelet transfusions were given and Prednisone was restarted at 2 mg/kg/day with a rise in platelet count to 170 000/mm He remained on low dose steroids (0 2 mg/kg/day) for one year without symptoms Platelet count varied but remained consistently well below normal In October 1966 it was 10 000/mm All steroids were discontinued for 4 weeks and in November 1966 azathioprine alone was begun at 2 mg/kg/day There was little response over a 10 week period Prednisone was restarted and maintained at 0 1 mg/kg/day with a response seen over the next 6 months Steroids were discontinued Platelet count fell with an upper respiratory infection but returned to good levels When azathioprine was discontinued after total 2 years therapy the platelet count promptly fell to 56 000 with an upper respiratory infection but without return of symptoms then rose to 180 000 held at 200 000/mm³ for 4 months and fell again to 36 000 He has been restarted on azathioprine 2 5 mg/kg/day and after 2 months of therapy his platelet count is 150 000/mm Although his course has been long and variable the response to therapy has been designated as good (Fig 5)

RESULTS

Table 1 summarizes the results of therapy The response to therapy is graded according to the platelet count and symptomatology as 1) Excellent complete hematological and clinical re

Table 1 *Azathioprine (imuran) therapy for refractory idiopathic thrombocytopenic purpura in childhood*

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1 P B	M	12	24 mo	Prednisone 2 mg/kg	40 000	220 000	2 5 mg	12	None	12	None	Excellent
2 C C	F	10½	23 mo	Prednisone 2 mg/kg	40 000	220 000	2 0–3 0 mg	12	None	3	None	Excellent
3 S D	F	4½	17 mo	Prednisone 2 mg/kg	50 000	250 000	2 0–5 0 mg	8	Splenectomy	12	None	Failure
4 E V	F	2½	5 yrs	Prednisone 2 mg/kg Splenectomy	10 000	210 000	2 5 mg	24	Prednisolone 0 2 mg/kg	3	None	Excellent
5 R K	M	2½	12½ yrs	Prednisone 2 mg/kg Splenectomy	< 10 000	200 000	2 5 mg	27	Prednisolone 0 2 mg/kg	4	Azathioprine 2 5 mg/kg/day	Good

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								SGPT 119 (nl 5–40 U/ml)	Normal
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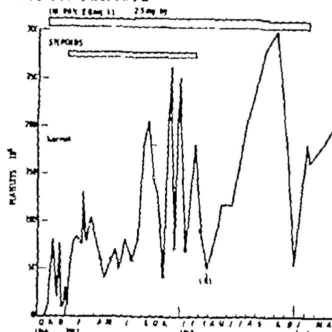


Fig 5 Course of disease in case 5 (R K B D 12/53 Splenectomy 5/61)

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5 R K	M	2½	1-½ yrs	Prednisone 2 mg/kg Splenectomy	< 10 000	200 000	2 5 mg	27	Prednisolone 0 2 mg/kg	4	Azathioprine 2 5 mg/kg/day	Good

vantageous and resulting in a reduction in the risk of infection since it may not be necessary to suppress all immune responses to obtain a therapeutic response. Immunoglobulins determined at the end of therapy in 3 of our patients were considered within normal levels for IgG with a slightly low level of IgM in one case. Infections were not increased in the total group nor in the patient with low IgM during the 30 months of follow. Therefore the dosage of drug used does not seem to cause irreversible damage to the bone marrow or liver or cause any harmful depression of immunologic responses in the child.

A recent review of the use of azathioprine with renal transplants by Penn *et al.* (9) reports the development of a malignant tumor in 5 of the total 120 patients transplanted. Antilymphocytic globulin had been given in 4 of these patients. Since oncogenesis is known to occur with a depression of immunologic reactivity by thymectomy, antilymphocytic globulin, azathioprine and chronic antigenic stimulation, the role of azathioprine in the development of neoplasia in the described cases is unclear. The use of azathioprine in nephrosis and other autoimmune diseases (1, 4, 6, 7, 8, 11, 13, 15) without the development of neoplasia suggests that a factor other than azathioprine was operative in the development of malignancy in the renal transplant patients.

SUMMARY

Five children *with refractory ITP were treated* with azathioprine for 8–27 months. An excellent response was obtained in 3 of the 5 patients, a good response in a *fourth*. In two pre-splenectomy cases the result was obtained using azathioprine alone and two post-splenectomy cases required low dose steroids in addition to azathioprine. One pre-splenectomy patient failed to respond to the drug alone or in combination with steroids but responded well to splenectomy. A review of the cases in the literature and the experience presented here suggests that azathioprine has a definite role in the therapy of refractory idiopathic thrombocytopenic p ur in childhood.

REFERENCES

1. Anesberg A C. Current concepts: drugs employed for suppression of immunologic responsiveness. *New Eng J Med 272* 1114, 1965.
2. Baldini M. Idiopathic thrombocytopenic purpura. *New Eng J Med 274* 1360, 1966.
3. Bouroncle B A & Doan C A. Refractory idiopathic thrombocytopenic purpura treated with azathioprine. *New Eng J Med 275* 630, 1966.
4. — Treatment of idiopathic thrombocytopenic purpura. *JAMA 207* 2049, 1969.
5. Cohen P & Gardner F H. Thrombocytopenic effect of sustained high dosage prednisone therapy in thrombocytopenic purpura. *New Eng J Med 265* 611, 1961.
6. Corley C C, Lessner H E & Larson W E. Azathioprine therapy of autoimmune diseases. *Am J Med 41* 404, 1966.
7. Kurzenko J A & Kesdan S E. Treatment of chronic idiopathic thrombocytopenic purpura with azathioprine and prednisolone: a clinical trial with three children. *Clin Ped 7* 216, 1968.
8. Mason M, Currey H L F, Barnes C G, Dunne J F, Hazleman B L & Strickland I D. Azathioprine in rheumatoid arthritis. *Brit Med J 1* 420, 1969.
9. Penn I, Hammond W, Brettschneider L & Starzl T E. Malignant lymphomas in transplantation patients. *Transplantation Proceedings 1* 106, 1969.
10. Rundles R W, Laszlo J, Itoga T, Hobson J B & Garrison F E Jr. Clinical and hematological study of 6 (1 methyl-4 nitro 5 imidazolyl) thiopurine (B W 57 322) and related compounds. *Cancer Chemotherapy Reports 14* 99, 1961.
11. Schwartz, R. & Dameshek W. Treatment of autoimmune hemolytic anemia with 6-mercaptopurine and thioguanine. *Blood 19* 483, 1962.
12. Smith C H, Erlandson M, Schulman I & Stern G. Hazard of severe infections in splenectomized infants and children. *Am J Med 22* 390, 1957.
13. Sussman L N. Azathioprine in refractory idiopathic thrombocytopenic purpura. *JAMA 202* 259, 1967.
14. Swanson M A & Schwartz, R S. Immunosuppressive therapy: the relation between clinical response and immunologic competence. *New Eng J Med 277* 163, 1967.
15. Theodor E, Gilon E & Waks U. Treatment of ulcerative colitis with azathioprine. *Brit Med J 1* 741, 1969.

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Key words: Thrombocytopenic purpura, children, azathioprine

Table 3 *Review of patients treated with azathioprine for chronic idiopathic purpura*

Result	No of patients	Authors	Ages
Excellent	15	Bouroncle & Doan	9 23 33 52 70 (4)
		Corley *et al*	64 (6)
		Kuzemko & Keidan	1½ 6 (7)
		Sussman	15 28 43 (13)
		Present report	2½ 10½ 12
Good	11	Bouroncle & Doan	23 42 50 53 56 59 70 (4)
		Corley *et al*	64 (6)
		Sussman	35 77 (13)
		Present report	2½
Fair	4	Bouroncle & Doan	42 55 (4)
		Sussman	53 54 (13)
Failure	7	Bouroncle & Doan	7 43 48 (4)
		Corley *et al*	39 (6)
		Kuzemko & Keidan	10 months (7)
		Sussman	44 (13)
		Present report	4½
Total	37		

shown to have a steroid sparing effect in certain diseases such as ulcerative colitis and rheumatoid arthritis Theodor *et al* (15) were able to achieve remission with the use of azathioprine in 4 of 7 patients with ulcerative colitis not controlled by steroids alone and demonstrated that steroids could be reduced in these patients Mason *et al* (8) showed a 35% reduction of steroid dosage in patients with rheumatoid arthritis given azathioprine without adverse side effects The dose of steroids used temporarily in 2 of our patients was low and is consistent with the findings of other investigators that azathioprine has a sparing effect on steroid dosage The toxic effects seen in the patients described in this paper have been transitory leukopenia minimal reduction in hemoglobin and alterations in liver transaminase levels All corrected when the drug was withdrawn

Review of the literature (Table 3) revealed 32 cases (27 adults and 5 children) with chronic recurrent ITP who have been treated with azathioprine (4 6 7 13) Of these 32 patients 12 had an excellent response 10 had a good response 4 a fair response and 6 had a poor response or failed to respond The present investigation of 5 children showed that 3 had an excellent result 1 had a good response whereas 1 unsplenectomized patient failed to respond but responded excellently to splenectomy Including the present investigation and patients of all ages 45% had an excellent response, 21% a good response 17% fair and 18% failed to respond to the use of azathioprine Response does not seem to be related to duration of disease or age of the patients Bouroncle & Doan (4) and Sussman (13) have indicated that prolonged therapy may have to be given for an adequate response Our patients were treated for at least 8 months before the combination of azathioprine plus low dosage steroids was considered a failure and therapy was continued for as long as 27 months for complete remission in a previously splenectomized patient

The relationship between clinical response and immunologic competence is important in the use of azathioprine Swanson & Schwartz (14) found inhibition of primary and secondary immune responses with delayed hypersensitivity during therapy with either azathioprine or amethopterin a decrease in IgG levels and normal synthesis of IgM The levels of IgG however appeared to be adequate in their patients for antiviral and antibacterial activities This selective immunosuppressive effect has been considered by these authors to be ad



Fig. 1. Interscapular telangiectases in a healthy girl aged 9 years.

cance in the closure of the medullary tube and he found these vascular telangiectases only at sites where the medullary tube had closed. These ectasias were thus instrumental in the covering of nerve tissue by skin and do not represent any error of development.

It must be mentioned, however, that Schnyder (21, 22) by means of equally thorough histological investigations of the skin corresponding to the naevi could not demonstrate ectasia of the vessels in infants but demonstrated moderate ectasia of the subpapillary vessels in older children.

Van der Werf (29) mentioned frontal, occipital and sacral types of naevi and was in agreement with Bossard and also influenced by Hochstetter's work in considering that the occurrence of the naevus was connected with the development of the medullary tube.

Theories concerning the mode of development have occasionally been quite unrealistic, for which reason Duxey (9) closed a discussion in the *Lancet* with the words "... any midwife will tell ... that it is caused by an affectionate peck from the stork who delivers the child before taking final leave of its charge." To this Bean (1) remarked that "this seems as good an idea as any."

Several authors (23, 29, 31) considered that hereditary factors must be of significance for the development of nuchal naevus but this presumption is contested by Sklarz (24) who considered that the naevus ... is so extremely frequent that the question of heredity seems to be of no significance. This is scarcely entirely justified.

Interscapular telangiectases (Fig. 1)

It was impossible to find interscapular telangiectases described in the current textbooks and handbooks. They are thus not mentioned in Rook's textbook of dermatology from 1968

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REVIEW ARTICLE

NUCHAL NAEVI AND INTERSCAPULAR TELANGIECTASES

Incidence in Danish School Children

JAKOB ØSTER and ARNE NIELSEN

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During an investigation of school children con cerning hæmangiomata attention was drawn to two phenomena viz the frequent incidence of pale red salmon-coloured naevi in the fovea nuchae level with the skin and most frequent ly localised above the hair margin and the frequent incidence of fine distended blood vessels or telangiectases in the superior interscapular region Both of these phenomena were surprising because the nuchal naevi so frequently observed in newly born infants, are said to disappear spontaneously according to current text books and general pædiatric opinion and be cause the interscapular region is not a generally recognised site for telangiectases Pædiatric lit erature on these cutaneous phenomena is ex tremely scanty After some search relevant literature mainly from dermatological sources was found

Nuchal naevi

In the literature nuchal naevi are mentioned under many names erythema nuchae capillary telangiectases telangiectatic naevus nuchae, haemangioma nuchae salmon patches of the nape naevus flammeus vinosus nuchae or merely the nape naevus which is termed Unna s naevus in many centres

As early as 1881 French physicians (1) appear to have described this naevus under the term tache sanguine but the German derma tologist Unna (27) in 1894 presented the first detailed description He mentioned in his book that Virchow had known this naevus and pres ented the conception that it developed in sites corresponding to the embryological clefts a theory which has never been generally accepted Unna considered that these nuchal naevi together with the corresponding naevi in the fore head region, were due to intrauterine pressure phenomena This theory has not found accept ance latterly one of the reasons being that sev eral authors (3 4) observed naevi in infants delivered by Caesarean section

As regards the origin Bossard (4) considered dass die Telangiektasien nichts anderes dar stellen als mangelhaft zuruckgebildete Bezirke aus dem erweiterten Gefassflecht der allmahlich vorruckende Vaskularisationszone In this opinion he was inspired by the investigations undertaken by the Viennese pathologist Hoch stetter (11) concerning the vascularization of the cranial skin in human foetuses Later Spraf ke (26) reached a similar conclusion from histological investigations of the skin of the nuchal region in foetuses children and adults He found that vascular telangiectases were par ticularly pronounced in the foetuses and inter preted nuchal naevi as resulting from persist ence of vascular formations which are of signifi



Fig. 1 Interscapular telangiectases in a healthy girl aged 9 years

cance in the closure of the medullary tube and he found these vascular telangiectases only at sites where the medullary tube had closed These ectasias were thus instrumental in the covering of nerve tissue by skin and do not represent any error of development

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Table 1 *Incidence of nuchal naevi and interscapular telangiectases in 2 171 Danish school children subdivided according to age and sex*

Telangiectases	Boys					Girls				
	+	+	–	–		+	+	–	–	
naevi	+	–	+	–	Total	+	–	+	–	Total
Age										
6	2	2	2	1	7	1	1	—	2	4
7	10	12	11	25	58	9	24	10	28	71
8	8	21	14	26	69	18	16	17	22	73
9	11	21	12	32	76	18	13	13	23	67
10	6	18	34	31	89	18	22	22	26	83
11	9	19	13	34	75	18	20	18	16	72
12	16	24	25	40	105	26	18	24	20	88
13	14	11	29	49	103	32	12	31	19	94
14	33	32	27	85	177	34	46	57	55	192
15	23	36	45	105	209	18	36	70	83	207
16	8	12	28	54	102	10	18	30	49	107
17	1	2	1	13	17	4	—	3	14	21
Total	141	210	241	495	1 087	206	226	295	357	1 084

(19) On the other hand Bean (1) mentioned in his unique work on vascular spiders and related lesions of the skin a phenomenon which he called the cervical fringe and which consists of readily visible veins in hair like whisps or linear strands at the base of the neck posteriorly They may extend down between the shoulderblades or occur only in these regions His attention had been drawn to the cervical fringe by Holt (12) who gave a good description in the *Lancet* in 1916 of what he called cervical capillary markings in the neighbourhood of the upper dorsal vertebrae

This communication resulted in a series of observations (6 16) from other authors who supported Holt in his interpretation of the had noticed the same phenomenon and who supported Holt in his interpretation of the phenomenon as a tuberculous manifestation Riviere (18) considered however that these telangiectases in some cases are very probably of naevoid nature and allied to the venules so often present in the red parts of the cheek' and Hughes (14) did not believe that the dilated blood vessels had any connection with tuberculosis but that they might possibly be caused by the pressure and friction of clothing over prominent or relatively unyielding parts and that constitutional peculiarities of the skin also probably play a part Finally the dermatologist Parkes Weber (28) concluded the discussion by drawing attention to the fact that he had described the phenomenon in 1904 and that, in his opinion it was not connected with tuberculosis His description appears to have been the first but Holt s description which was made independently of Parks Weber is the best

MATERIAL AND METHOD

During the school session 1967 68 one of the authors (J Ø) undertook a systematic investigation concerning nuchal naevi and interscapular telangiectases in 2 171 school children aged 6–17 years attending three schools in the town of Randers Boys and girls were approximately equally represented The material is not selected and includes children from both the academic and ordinary classes All of the children were investigated for the above mentioned conditions and in addition an ordinary clinical investigation was undertaken The great majority of children were examined in daylight and it must be emphasized that naevi of the above mentioned type which in the great majority of cases are localized to the skin above the hair margin may be particularly difficult to detect particularly in boys with short thick hair The telangiectases are most easily seen when the child is requested to press the shoulders forward so that the skin in this region is stretched There is thus a possibility of clinical errors in diagnosis which make the relevance of clinical assessment debatable Minimum figures are probably relevant

Table 2 Incidence of nuchal naevi and interscapular telangiectases in Danish school children

	Girls	Boys	Total
Nuchal naevi	501	382	883
Interscapular telangiectases			
Slight	317	269	586
Moderate	103	72	175
Pronounced	12	10	22
Total	432	351	783
out of	1 084	1 087	2 171
Percentage with naevi	46 2	35 1	40 7
Percentage with telangiectases	39 9	32.3	36 1

RESULTS

The entire material is recorded in Table 1 and comparison of the incidence of nuchal naevi and telangiectases in the interscapular region is given in Table 2

In order to investigate whether there is any connection between the two conditions the incidence of naevi expressed as a percentage of the individuals with and without telangiectases was calculated for each age group and sex The results are apparent from Fig. 2 *a* for boys and Fig 2 *b* for girls where the abscissa gives the age and the ordinate the incidence of the naevus In addition two horizontal lines indicate the incidence of naevi for all age groups together and for each of the two groups of individuals with and without telangiectases respectively

Where boys are concerned the incidence of naevi is greater in nine out of the 12 age groups among those who have telangiectases than among those without and lower in the remaining three groups

Where girls are concerned the incidence of naevi is greater in seven out of the 12 age groups among those with telangiectases than among those without and lower in the remaining five groups

Significant differences between the incidences were found in the following sex and age groups Boys aged 10 years significance at 5 per cent level lower incidence of naevi among those with telangiectases than without boys aged 14 years significance at 0 1 per cent level higher incidence of naevi among those with telangiectases than those without girls aged 17 years significance at 5 per cent level higher incidence of naevi among those with telangiectases than among those without Taken as a whole the material provides no support for the presumption that there is any connection between the incidence of naevi and telangiectases

In Fig 3 *a* the incidence of naevi is expressed as a percentage as compared with the age the sexes being considered separately and in

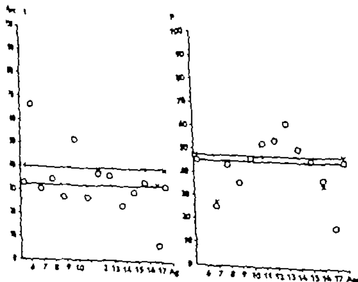


Fig 2 (a) Boys (b) Girls Percentage with naevus according to age among ○ schoolchildren with telangiectases ○ schoolchildren without telangiectases Horizontal line indicates overall percentage for all ages

Table 1 Incidence of nuchal naevi and interscapular telangiectases in 2 171 Danish school children subdivided according to age and sex

Telangiectases naevi	Boys + +	+ −	− +	− −	Total	Girls + +	+ −	− +	− −	Total
Age										
6	2	2	2	1	7	1	1	—	2	4
7	10	12	11	25	58	9	24	10	28	71
8	8	21	14	26	69	18	16	17	22	73
9	11	21	12	32	76	18	13	13	23	67
10	6	18	34	31	89	18	22	22	26	88
11	9	19	13	34	75	18	20	18	16	72
12	16	24	25	40	105	26	18	24	20	88
13	14	11	29	49	103	32	12	31	19	94
14	33	32	27	85	177	34	46	57	55	197
15	23	36	45	105	209	18	36	70	83	203
16	8	12	28	34	102	10	18	30	49	107
17	1	2	1	13	17	4	—	3	14	21
Total	141	210	241	495	1 087	206	226	295	357	1 084

(19) On the other hand Bean (1) mentioned in his unique work on vascular spiders and *related lesions of the skin* a phenomenon which he called the cervical fringe and which consists of readily visible veins in hair like whisps or linear strands at the base of the neck posteriorly They may extend down between the shoulderblades or occur only in these regions His attention had been drawn to the cervical fringe by Holt (12) who gave a good description in the *Lancet* in 1916 of what he called cervical capillary markings in the neighbourhood of the upper dorsal vertebrae

This communication resulted in a series of observations (6 16) from other authors who supported Holt in his interpretation of the had noticed the same phenomenon and who supported Holt in his interpretation of the phenomenon as a tuberculous manifestation Riviere (18) considered, however that these telangiectases in some cases are very probably of naevoid nature and allied to the venules so often present in the red parts of the cheek and Hughes (14) did not believe that the dilated blood vessels had any connection with tuberculosis but that they might possibly be caused by the pressure and friction of clothing over prominent or relatively unyielding parts" and that constitutional peculiarities of the skin also probably play a part Finally the dermatologist Parkes Weber (28) concluded the discussion by drawing attention to the fact that he had described the phenomenon in 1904 and that in his opinion, it was not connected with tuberculosis His description appears to have been the first but Holt s description which was made independently of Parks Weber is the best

MATERIAL AND METHOD

During the school session 1967 68 one of the authors (J Ø) undertook a systematic investigation concerning nuchal naevi and interscapular telangiectases in 2 171 school children aged 6–17 years attending three schools in the town of Randers Boys and girls were approximately equally represented The material is not selected and includes children from both the academic and ordinary classes All of the children were investigated for the above mentioned conditions and in addition an ordinary clinical investigation was undertaken The great majority of children were examined in daylight and it must be emphasized that naevi of the above mentioned type which in the great majority of cases are localised to the skin above the hair margin may be particularly difficult to detect particularly in boys with short thick hair The telangiectases are most easily seen when the child is requested to press the shoulders forward so that the skin in this region is stretched There is thus a possibility of clinical errors in diagnosis which make the relevance of clinical assessment debatable Minimum figures are probably relevant

Table 3 *Investigations of incidence of nuchal naevi*

The incidences are given as percentages and the figures in brackets are absolute figures

	Newly born infants			School children			Adults		
	Boys	Girls	Total	Boys	Girls	Total	Males	Females	Total
Jaat 1894 (77)									10-20
Saalfeld, 1910 (20)									57 (400)
Bossard 1918 (4)	46 5	54	50 (330)	12	40 5	27 (304)	15 5	24 5	20 (431)
Bardhausen 1928 (10)						8	3	25	
Hetzmaier 1930 (13)				4 3	21 4	22 8 (246)	24 7	26 8	25 8 (400)
Carson 1934 (8)			2 8 (210)						5 7 (175)
Spalke 1937 (26)				50	50	50	38	35	
Beatty 1940 (3)			62 (152)						50 (60)
van der Werf 1952 (29)				40 (788)	44 (233)	4. (521)		46 (152)	
Pratt, 1953 (17)			42 (879)						
Zuckeller 1957 (31)									50 (600)
Smith & Manfield 196.. (25)			40 (242)						20
Oster & Nielsen 1970				35 1 (1 087)	46 2 (1 084)	40 7 (2 171)			

same in the two groups in each separate age group It must therefore be presumed that the incidence of telangiectases in these age groups is greater in girls than in boys

DISCUSSION

Nuchal naevi

A comparison of the investigations of incidence which includes the present one is presented in Table 3 As it will appear from Table 3 the incidences *found* vary greatly not only within the same category but also in different categories e g newly born infants school children and adults respectively The first difference may be due to the fact that the number of individuals investigated has varied greatly and in several instances the absolute figures are not mentioned Another reason may be that investigation in certain children is rendered difficult by prolific growth of hair and finally the extent and form of the naevi may vary from small discrete patches to large confluent areas In the present work all cases were included ir respective of size or form This may be why the incidence is reported so differently in different age groups Bossard s work (4) which i the most thorough in this respect suggests that the incidence decreases throughout the various age groups from newly born infants to adults

Table 4 *Incidence of skin conditions of the scalp and nuchal region in children with nuchal naevi*

	Girls	Boys	Total
Naevus localized scaling	12	3	15
Naevus generalized pityriasis of scalp	4	1	5
Naevus nuchal neurodermatitis		0	2
Naevus psoriasis of scalp	0	1	1
Total	18	5	25

As regards school children there is good agreement between the incidences found in the present investigation and those found by van der Werf (29) which were also based upon an extensive material We are unable to explain the deviations attributable to age found in girls in the present investigation with an increase in incidence at the ages of 12–13 years

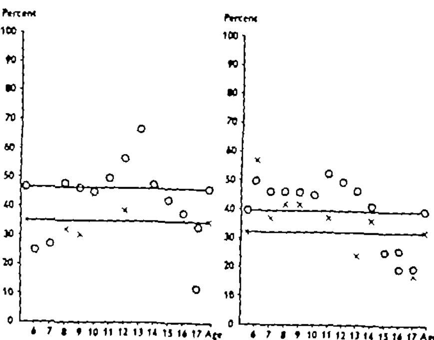


Fig 3 (a) Percentage with naevi among schoolchildren according to age (b) Percentage with telangiectases among schoolchildren according to age × boys ○ girls ⊙ and ○ significant deviation from "overall" percentage. Horizontal line indicates "overall" percentage for all ages

milarly in Fig 3 b the incidence of telangiectases are expressed as percentage as compared with age the sexes being considered separately In addition in both Figs the incidence for all age groups is indicated by two horizontal lines one for each sex

Calculations of significance were undertaken for whether the variations for a given sex were greater in the various age groups than might be anticipated from the binomial distribution if the incidence in the population was the same in all age groups (In the test the incidences h were converted to the magnitude 2 arcsin h whereupon the distribution of this magnitude is approximated with the normal distribution) As regards the incidence of naevi in boys no significance as regards the 5 per cent level was found while for girls significance for the 1 per cent level was found As regards the incidence of telangiectases significance as regards the 0 1 per cent level was found for both girls and boys The particularly deviant values are indicated by extra circles on the legends in Figs 3 a and 3 b in order to elucidate possible systematic examination

For boys with naevi the value at the age of 17 years is rather low and the variation around the average value gives the impression of being slightly greater than would be anticipated from binomial distribution although this was not significantly greater The values do not provide evidence against the presumption that the incidence is independent of age for boys in the age groups concerned

For girls with naevi there is significant deviation downwards at the age of 7 years, upwards at the ages of 12 and 13 years and downwards at the age of 16 years This suggests a variation due to age with an increase at the ages of 12–13 years and a subsequent decrease to more or less the same level The values at the age of 6 years (4 children) and 7 years (71 children) are however low compared with the subsequent values

For boys and girls with telangiectases the course is more or less parallel The presumption of constant values up to the age of 14 years with a subsequent decrease where both sexes were concerned appears to be reasonable taking into consideration the particularly deviant values in calculation of significance Apart from the values at the age of 6 years (only 7 boys and 4 girls) the incidence in girls of all age groups in the horizontal plane (6–14 years) was greater than the incidence in boys The probability that the sign for the difference in cidence in girls minus incidence in boys in one or none of the nine age groups should be the opposite of that in the remaining groups is only 3 9 per cent provided that the incidence is the

Table 3. *Investigations of incidence of nuchal naevi*
The incidences are given in percentages, and the figures in brackets are absolute figures

	Newly born infants			School children			Adults		
	Boys	Girls	Total	Boys	Girls	Total	Males	Females	Total
Unna 1894 (27)									10–20
Saalfeld 1910 (20)									57 (400)
Bostard 1918 (4)	46.5	54	50 (330)	12	40.5	27 (304)	15.5	24.5	20 (431)
Havthausen 1928 (10)						8	3	25	
Holzammer 1930 (13)				4.3	21.4	22.8 (246)	24.7	26.8	25.8 (400)
Coson 1934 (8)			2.8 (210)						5.7 (175)
Sprafke 1937 (26)				50	50	50	38	55	50 (60)
Bentley 1940 (3)			62 (15.)						
van der Werf 1952 (29)				40 (288)	44 (233)	42 (571)		46 (152)	
Pratti 1953 (17)			4 (879)						
Zunkeller 1957 (31)									50 (600)
Smith & Manfield 1962 (25)			40 (247)						20
Oster & Nielsen 1970				35.1 (1,087)	46.2 (1,084)	40.7 (2,171)			

same in the two groups in each separate age group. It must therefore be presumed that the incidence of telangiectases in these age groups is greater in girls than in boys.

DISCUSSION

Nuchal naevi

A comparison of the investigations of incidence which includes the present one is presented in Table 3. As it will appear from Table 3, the incidences found vary greatly not only within the same category but also in different categories, e.g. newly born infants, school children and adults, respectively. The first difference may be due to the fact that the number of individuals investigated has varied greatly and in several instances the absolute figures are not mentioned. Another reason may be that investigation in certain children is rendered difficult by prolific growth of hair and finally the extent and form of the naevi may vary from small discrete patches to large confluent areas. In the present work all cases were included irrespective of size or form. This may be why the incidence is reported so differently in different age groups. Bostard's work (4), which is the most thorough in this respect, suggests that the incidence decreases throughout the various age groups from newly born infants to adults.

Table 4. *Incidence of skin conditions of the scalp and nuchal region in children with nuchal naevi*

	Girls	Boys	Total
Naevus + localized itching	12	3	15
Naevus + generalized pityriasis of scalp	4	1	5
Naevus + nuchal neurodermatitis	2	0	2
Naevus + psoriasis of scalp	0	1	1
Total	18	5	25

As regards school children, there is good agreement between the incidences found in the present investigation and those found by van der Werf (29) which were also based upon an extensive material. We are unable to explain the deviations attributable to age found in girls in the present investigation with an increase in incidence at the ages of 12–13 years.

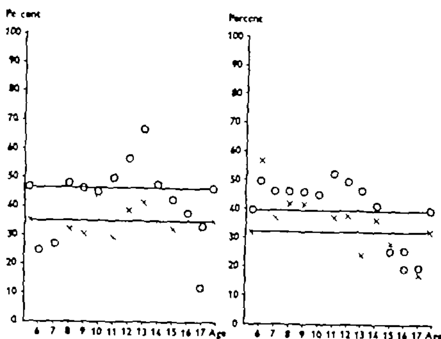


Fig 3 (*a*) Percentage with naevus among schoolchildren according to age (*b*) Percentage with telangiectases among schoolchildren according to age boys O girls ⊗ and O significant deviation from overall percentage Horizontal line indicates overall percentage for all ages

milarly in Fig 3 *b* the incidence of telangiectases are expressed as percentage as compared with age the sexes being considered separately In addition in both Figs the incidence for all age groups is indicated by two horizontal lines one for each sex

Calculations of significance were undertaken for whether the variations for a given sex were greater in the various age groups than might be anticipated from the binomial distribution if the incidence in the population was the same in all age groups (In the test the incidences *h* were converted to the magnitude 2 arcsin *h* whereupon the distribution of this magnitude is approximated with the normal distribution) As regards the incidence of naevi in boys no significance as regards the 5 per cent level was found while for girls significance for the 1 per cent level was found As regards the incidence of telangiectases significance as regards the 0 1 per cent level was found for both girls and boys The particularly deviant values are indicated by extra circles on the legends in Figs 3 *a* and 3 *b* in order to elucidate possible systematic examination

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For boys and girls with telangiectases the course is more or less parallel The presumption of constant values up to the age of 14 years with a subsequent decrease where both sexes were concerned appears to be reasonable taking into consideration the particularly deviant values in calculation of significance Apart from the values at the age of 6 years (only 7 boys and 4 girls) the *incidence in girls* of all age groups in the horizontal plane (6–14 years) was greater than the incidence in boys The probability that the sign for the difference in incidence in girls minus incidence in boys in one or none of the nine age groups should be the opposite of that in the remaining groups is only 3 9 per cent, provided that the incidence is the

ders thorax cheeks and/or ears Among the 1 388 children without telangiectases in the interscapular region only 4 (2 girls and 2 boys) had similar telangiectases elsewhere

SUMMARY

Naevi in the fovea nuchae and telangiectases in the superior interscapular region have been found with great frequency in 2 171 school children aged 6–17 years during a systematic search for these phenomena The incidence of nuchal naevi was 46 2 per cent in girls 35 1 per cent in boys The incidence of telangiectasis was 39 9 per cent in girls and 32 3 per cent in boys

No connection was found between the incidence of naevi and telangiectases

Concerning nuchal naevi the incidence seems to be independent of age for boys while for girls there seems to be an increase at the ages of 12–13 years and a subsequent decrease

For boys and girls with interscapular telangiectases the course is parallel with constant values up to the age of 14 years with a subsequent decrease

A comparison is made of other investigations concerning these phenomena the significance of which is discussed

REFERENCES

1 Bean W B *Vascular spiders and related lesions of the skin* Charles C Thomas Springfield Ill 1958
2 Besnier E & Doyon A *Leçons sur les maladies de la peau* Masson Paris 1881
3 Bertley F R Erythema nuchae *Brit J Derm* 5 363 1940
4 Bossard Y Die blassen Feuermale der Kinder *Jahrb f Kinderh* 88 204 1918
5 Brock W Zur Frage der Entstehung der Nävi *Derm Ztschr Z* 47 184 1926
6 Chisholm C The significance of cervical capillary markings *Lancet I* 797 1916
7 Cleveland D E H Lichen simplex chronicus *Arch Derm* 33 316 1936
8 Cornoe E F Naevus flammeus nuchae its occurrence and abnormalities *Am J Med Sci* 187 121 1934
9 Drury I R B The Nape Naevus *Brit Med J* I 103 1955
10 Haxthausen H Teleangiektasien im Nacken *Derm Wschr* 86 479 1928
11 Hochstetter F *Über die Vascularisation der Haut des Schädeldaches menschlichen Embryonen* Denkschriften der Kaiserlichen Akademie der Wissenschaften Vol 93 Alfred Holder Wien 1916
12 Holt R C The significance of cervical capillary markings *Lancet I* 534 1916
13 Holzammer H *Nackenflecke* Inaug Dissert Erlangen 1933
14 Hughes E The significance of cervical capillary markings *Lancet I* 797 1916
15 Lynch F W Suboccipital dermatitis *Arch Derm* 60 307 1949
16 Meachen C N The significance of cervical capillary markings *Lancet I* 589 1916
17 Pratt A G Birthmarks in infants *Arch Derm* 67 302 1953
18 Riviere C The significance of cervical capillary markings *Lancet I* 697 1916
19 Rook A Wilkinson D S & Ebling F J G *Textbook of Dermatology* Blackwell Oxford & Edinburgh 1968
20 Saalefeld E Über Naevi angiomatosi in der Hinterhaupts Nackengegend *Med Klin* 6 145 1910
21 Schnyder V W Zur Klinik und Histologie der Angiome *Arch Derm* 198 51 1954
22 Schnyder V W Zur Klinik und Histologie der Angiome *Arch Derm* 200 483 1955
23 Shafar J & Doig A The Nape Naevus *Brit Med J I* 1221 1955
24 Sklarz E Telangiectatic ("Nape") Naevi *Brit Med J I* 1221 1955
25 Smith M A & Manfield P A The natural history of salmon patches in the first year of life *Brit J Derm* 74 31 1962
26 Sprafke H Klinische und histologische Untersuchungen über den Hinterhauptnackennaevus (Naevus Unna) und seine Beziehungen zur Spina bifida *Arch Derm* 175 168 1937
27 Unna G P *Die Histopathologie der Hautkrankheiten* Verlag von August Hirschwald Berlin 1894
28 Weber F Parkes The significance of cervical capillary markings *Lancet I* 599 1916
29 Werf E van der *Een Onderzoek naar Huidangiomen bij Schoolkinderen* Thesis Amsterdam 1952
30 Wiehl R Die Nackenhaut in ihrer besonderen neutralen Reaktionsfähigkeit *Z Haut Geschlecht skr* 18 61 1955
31 Zumkeller R A propos de la fréquence et de l'hérédité du Naevus Vasculosus Nuchae (Unna) *J Genét Hum* 6 1 1957

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As regards the significance of naevi it may be mentioned that Unna s (27) interest in nuchal naevi was aroused during the treatment of pityriasis of the scalp where a red patch frequently persisted despite intensive treatment Corson (8) mentioned that the skin over nuchal naevi frequently was affected by dermatitis, seborrheic or ezcematoid and Wiehl (30) also mentioned the connetcion between Unna s naevus and eczema of the nape

Various authors (7 15) have emphasized that the nuchal region is the site of predilection for neurodermatitis (lichen simplex) and that this condition in this site only occurs in women or usually is almost confined to women (19) Lynch (15) considered that the most suitable name for lichen simplex chronicus of the nape would be suboccipital dermatitis and based this on the fact that next to itching redness was the commonest clinical feature and was usually associated with thickening and frequently scaling in most instances the plaque was practically limited to the fossa He considered that the inflammatory reaction at this site is more pronounced than in lichenization elsewhere and has observed histologically that capillary dilatation was commonly present, and was frequently associated with congestion He did not mention any connection between nuchal naevi and lichen of the nape Nevertheless it was in fact this connection which he had observed clinically and histologically In his attempt to explain lichen of the nape and its predominance in women as caused by endocrine disturbances he had in fact touched on this connection in his reference that endocrine agents often manifest selectivity of site of action usually influenced by anatomic structure but sometimes by way of vasomotor phenomena

It is apparent from Table 4 that in the present material there were 15 children with nuchal naevi the majority of whom were girls who had localised scaling corresponding to the naevus and 2 cases both girls had neurodermatitis in the same region Further a girl aged 16 years with eczema and nuchal neurodermatitis but without naevi was found and also a boy aged 8 years with generalized eczema and nuchal naevus but with normal skin over the naevus In two girls aged 15 years who had no nuchal naevi, naevus flammeus of a darker colour than is seen in nuchal naevus localised to the skin between the scapulae in both over the left scapula in one and over the right hip region in the other No other diseases of the skin or hair were encountered in the children

Nuchal naevi thus appear to be of significance in the development of pityriasis localised to the same region while on the other hand, it is more doubtful whether there is a connection between nuchal naevi and neurodermatitis in the age group investigated

Interscapular telangiectases

Riviere (18) in a material of 164 healthy school children found these dilated venules in 71 (43 3 per cent) These were strongly marked in 16 slight in 30 and merely present" in 25 Later Bean (1) mentioned that the relation to age is more obvious in men for I have observed a number of young women in the late teens and twenties with them though they are commoner in elderly women In men they are rare before the fifties

The incidence among the school children investigated here is of the same magnitude as in Riviere s work But as is apparent from Table 2, in the vast majority of the cases the telangiectases are only slightly pronounced and may easily be overlooked if not particularly looked for In contrast to Bean s remarks the present authors found a constant incidence up to the age of 14 years always slightly higher in girls than in boys and thereafter a steady decrease in both sexes

The genesis and significance are obscure Tuberculosis and other pulmonary disease may be excluded as having any relationship to interscapular telangiectases

It may be noted that among the 783 children with telangiectases in the interscapular region there were 10 (6 girls and 4 boys) with pronounced telangiectases elsewhere on the shoul

Table 1 *Fluoride content of infant food*
Estimated average values. Dry milk formulas S and F denote two of the most popular brands on the Swedish market

	Proportion	F content mg/kg mg/l	F content in relation to breastmilk
Breastmilk		0.025	
Cow's milk		0.03	
Cow's milk + water 0.25 ppm F	1 + 1	0.14	5.5
Cow's milk + water 1 ppm F	1 + 1	0.57	21
Cow's milk + water 5 ppm F	1 + 1	2.52	100
Dry milk formula S		0.40	
Dry milk formula S + water 0.25 ppm F	1 + 6	0.32	13
Dry milk formula S + water 1 ppm F	1 + 6	1.07	43
Dry milk formula S + water 5 ppm F	1 + 6	5.07	200
Dry milk formula F		5.0	
Dry milk formula F + water 0.25 ppm F	1 + 6	1.05	42
Dry milk formula F + water 1 ppm F	1 + 6	1.8	72
Dry milk formula F + water 5 ppm F	1 + 6	5.8	230

Table 2 *Classification of enamel fluorosis*
Individual index is determined by the most pronounced type found in two teeth. Group index is the average of individual index numbers

	Index
Normal enamel	0
Questionable fluorosis (very small white dots)	0.5
Very mild fluorosis (small opaque spots distributed over less than 25% of the surface)	1
Mild fluorosis (larger white spots covering less than 50% of the surface)	2
Moderate fluorosis (the whole surface affected, often brown discolouration)	3
Severe fluorosis (the whole surface affected, brown discolouration, hypoplastic enamel)	4

dren as regards the parameters of enamel fluorosis must imply a wide latitude of tolerance to fluoride supply during the first year of life. All previous experience indicates that skeletal fluorosis—the main health hazard of excessive fluoride ingestion—is not found in a population as long as enamel fluorosis does not reach high average index values (5).

It may be questioned whether ordinary drinking waters contain substances other than fluorine which can be supplied in greatly increased quantity with formula feeding. Manganese is theoretically most likely since its concentration in drinking water may be more than 50 times as high as in human milk where it has been reported to be 7 μg/l (1). However, no data on the toxicity of manganese seem to cause any concern in this respect (6).

SUMMARY

Infant feeding with water-diluted cow's milk or still more dry milk formulas supplies many

Table 3 *Distribution of enamel fluorosis in Uppsala children* ($M \pm S.E.$)

		Index	Mean no. of affected 1st perm. molars/child	Mean no. of affected perm. upper central incisors/child
All B-children All F-children	131 129	1.02 ± 0.06 1.17 ± 0.07 $t = 1.60$ $p > 0.1$	2.40 ± 0.15 2.64 ± 0.13 $\chi^2 =$ [illegible]60, DF = 4 $p > 0.1$	1.24 ± 0.08 1.43 ± 0.07 $\chi^2 = 2.01$, DF = 2 $p > 0.3$
B-children F-children	18 74	1.00 ± 0.20 1.17 ± 0.10 $t = 0.783$ $p = —$	[illegible]28 ± 0.40 2.[illegible] ± 0.16 $\chi^2 = 4$.[illegible], DF = 4 $p = —$	1.17 ± 0.23 1.42 ± 0.10 $\chi^2 = 1.512$, DF = 2 $p = —$

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SHORT COMMUNICATION

INCREASED FLUORIDE INGESTION BY BOTTLE FED INFANTS AND ITS EFFECT

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It has recently been found that the fluoride content of breastmilk is well below 0 05 ppm much less than previously accepted and about the same as the ionised fluoride content of plasma and the fluoride content of saliva and hardly appears to be influenced by the mother s fluoride ingestion (4) This low fluoride con tent of the breastmilk implies a many times smaller fluoride dose than that obtained with the dry milk feeding which has become domi nant in later years both in Sweden and in many other countries Calculated fluoride doses ap pear in Table 1

Since these variations of fluoride supply coin cide with a period when the enamel of many of the permanent teeth is mineralizing the first symptom to be expected if any should be an increased frequency and degree of enamel fluo rosis The purpose of the present investigation has been to compare the occurrence and de gree of enamel fluorosis in schoolchildren whose diet as infants has been dominated by either breastmilk or water diluted dry milk formulas

MATERIAL AND METHODS

The main investigation was carried out on 8–9 year old children in Uppsala This city has for many years had a natural content of about 1 2 ppm F in the drinking water which means that a formula fed child in Uppsala will ingest at least *50 times more fluoride* than a breast fed child

Two groups of children were selected for the examinations on the basis of a detailed questionnaire to the parents

1 children who were *breast fed for at least 5* months with no period of exclusive feeding with water diluted dry milk preparations (B children)

2 children who received exclusively water-diluted dry milk formulas for at least 5 months (F children)

Later a sub classification was made in order to compare still more extreme groups As B+ children were registered those B children for whom no period of even partial feeding with dry milk formulas was *reported* and as *F+ children* those F children who received water diluted dry milk formulas exclusively for at least 9 months of their first year of life

The examination of the children was carried out during the autumn term 1969 by one of the authors (U R) who did not know the group classification of the children Enamel fluorosis was classified accord ing to Dean (2 3 see Table 2) Cases which appeared intermediate according to this scheme were classified as *1 5 2 5 or 3 5*

RESULTS

Average values of enamel fluorosis index and numbers of mottled first permanent molars and permanent upper central incisors appear in *Table 3 No significant difference* existed even between the selected groups B+ and F+ but there is throughout a tendency to more pro nounced enamel fluorosis in the F children

DISCUSSION

The absence of any significant differences be tween typically breastfed and formula fed chil

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CASE REPORT

NEONATAL MENINGITIS CAUSED BY SALMONELLA THOMPSON

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The cause of purulent meningitis in the newborn period may b any of the common pathogens of meningitis in later life but it is well known that the infection is caused mostly by gram negative bacilli belonging to the colon group of organisms Of these the *E coli* is by far the most common but typhoid bacillus and various types of th salmonella group have also been reported as causative agents Purulent meningitis caused by salmonella organisms is relatively rare (7 10 11) but deserves nevertheless a certain attention as its treatment may involve complex therapeutic problems in the newborn period The purpose of this case report is to emphasize some of these problems

CASE REPORT

The patient a girl was born Sept. 11 1967 23 days after expected confinement Pregnancy and delivery were without complications Birth weight 3 010 g and length 49 cm During the first 9 days of life nothing abnormal was observed The girl was nursed by the mother without difficulties and had started to gain weight when she fell ill on the 10th day with high fever She was admitted to hospital on the same day and showed a slightly impaired general condition and signs of light dehydration The cerebrospinal fluid was turbid and contained 700 cells/mm³ mostly polynuclear leucocytes the protein content was 528 mg 100 ml and sugar 97 mg/100 ml Direct microscopy revealed gram negative bacilli and on the suspicion that the infection was caused by *E coli* parenteral treatment with a combination of cephalothin benzyl penicillin and sulfisomidine (700 mg/kg body weight/24 h) was instituted The following day cephalothin was exchanged for oxytetracycline (50 mg/kg body weight/24 h) and ampicillin (170 mg kg body weight/24 h) was introduced instead of benzyl penicillin (Fig 1) Culture of the cerebrospinal fluid yielded an organism belonging to the salmonella group and was identified as *S Thompson* Cultures of blood and stool were also positive for S Thompson The organism was highly sensitive to oxytetracycline ampicillin and sulfisomidine and after treatment for 60 hours the patient's temperature was normal A new lumbar puncture was performed after 6 days of treatment but culture of the cerebrospinal fluid still demonstrated growth of S Thompson Oxytetracycline was then *exchanged* for chloramphenicol which was given for 11 days (total amount 2 8 g) The treatment with ampicillin and sulfisomidine was continued for 1 month During this period repeated cultures of the cerebrospinal fluid were reported sterile and the number of leucocytes decreased to 41/mm³ with only a few neutrophils The protein and sugar content of the cerebrospinal fluid decreased to 120 mg/100 ml and 41 mg 100 ml respectively Chlorides were 119 mEq l The infant's general condition improved and she gained weight.

Four days after all treatment had been discontinued the patient started to vomit and ran a high fever Lumbar puncture revealed a turbid cerebrospinal fluid with 3,300 cells/mm³ mostly neutrophil leucocytes Growth of *S Thompson* was demonstrated but the blood culture was this time sterile Treatment with streptomycin *sulfate* ampicillin and sulfisomidine was immediately instituted Streptomycin was given for 8 days (total dose 1 04 g) and during this time three blood transfusions were given with a few days interval It was decided to continue with ampicillin and sulfisomidine for 3 months and during this time the cerebrospinal fluid became normal all further cultures were sterile and the infant thrived and developed quite normally Hydrocephalus or other signs of neurological abnormalities have not been observed during the follow up period of 2 years Repeated electroencephalograms and echoencephalograms have not revealed anything abnormal

The mother was quite healthy during pregnancy and after delivery but stool cultures taken one day

times greater fluoride doses than breast feeding already with low water fluoride content An investigation of schoolchildren in Uppsala (1 2 ppm F in the drinking water) showed only an insignificant trend towards increased enamel mottling in typically formula fed children compared to breast fed children No health hazard thus seems to be involved

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REFERENCES

1 Brock A & Wolff L K The quantity of Cu Zn and Mn in cow s milk *Acta Brev Neerl Physiol Pharmacol Microbiol* *5* 80 1935
2 Dean H T Classification of mottled enamel diagnosis *J Am Dent Ass* *21* 1421 1934
3 — The investigation of physiological effects by the epidemiological method *in* F R Moulton (ed) *Fluorine and dental health* Am Ass Adv Sci Washington D C 1942
4 Ericsson Y Fluoride excretion in human saliva and milk *Caries Res* *3* 159 1969
5 Hodge H C & Smith F *Fluorine chemistry* vol IV Academic Press New York 1965
6 Underwood J E *Trace elements in human and animal nutrition* Academic Press New York and London 1962

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Table 2 *Immunoglobulin levels in mg/100 ml*

	IgG		IgA		IgM	
Age	Patient	Normal mean and range	Patient	Normal mean and range	Patient	Normal mean and range
3 weeks	360	960 585–1133	2	3 5 1 8–6 2	50	33 4 13 2 67 0
5 weeks	360	—	4	—	32	—
6 weeks	390	702 510–846	17	7 4 1 7–12 7	50	31 1 10 7 57 1
2 months	460	—	48	—	81	—
3 months	330	432 278–672	24	12 2 7–28 2	48	37 1 14 9–68 1
3½ months	300	—	30	—	48	—
4½ months	310	367 231 578	30	15 2.9–37 7	27	36 8 18 6–56 7
6 months	290	470 2 0–645	30	14 2 4 4–35 8	70	43 8 18 6–69 6
years	370	705 453–1125	190	15 8 13 8–65 5	51	59 6 35 5–96 8

Normal values taken from Berg and Johansson & Berg (5 9)

of the microorganism this combination of drugs ought to have been highly effective In spite of 6 days treatment with adequate doses the cerebrospinal fluid did not become sterile which prompted the exchange of oxytetracycline for chloramphenicol Whether this change of therapy had any influence on the further outcome is an open question

The optimal duration of treatment of salmonella meningitis is not known (13) but a period of 2–3 weeks after symptoms have subsided is recommended (11–14) In the present case the infant was treated for 4 weeks but nevertheless a relapse occurred which indicates that the recommended duration of treatment may at least in some cases be too short Generally accepted criteria of successful treatment of purulent meningitis e g patient afebrile for 5 days cerebrospinal fluid cell count of 30 cells or less and normal sugar and protein content (12) appear to be of questionable value in a case of the type reported here Whether a treatment for 3 months as employed in the present case is really necessary seems impossible to decide but it may be concluded that when a non toxic drug as ampicillin can be used it is wise to continue the treatment for longer than the usually recommended 4 weeks

Investigations in recent years have given ample evidence that the newborn infant is capable of an immune response to most infectious agents (2) The response to salmonella antigens has been studied *in detail* (2 4) and the newborn infant is apparently able to produce agglutinins against the O antigens as well as the H antigens In the case presented here only antibodies to *S Thompson* O and OH antigens could be demonstrated and the titers did not reach very high levels (Table 1) The observed titers and the negative Widal test 2 years after the infection must however be taken as evidence of an immunological response Whether this response can be regarded as entirely normal is difficult to decide with certainty as *controls* with the specific antigen are lacking An evaluation of the results is also complicated by the three blood transfusions in the beginning of the second month of treatment

The rise in the titer observed at 6 months of age is best explained as an anamnestic reaction caused by colonization of the intestine with coli bacilli after discontinuation of the antibiotic treatment

The immunoglobulin levels were first measured during the second week of treatment

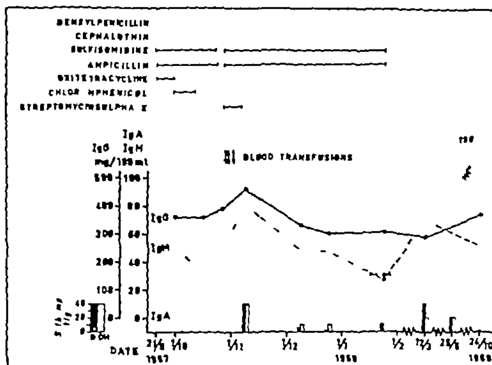


Fig 1

after admittance of the patient showed her to be a carrier of *S Thompson* The same pattern of resistance was demonstrated as shown by the organism isolated from her baby She has agglutinating antibodies to *S paratyphi B* O antigen and to *S Thompson* OH antigen corresponding to a previous salmonella infection Her immunoglobulin levels are within the normal range

During the course of the disease and for several months afterwards the Widal test and the immunoglobulin levels were followed The immunoglobulin determinations were performed with Oudin tube technique as described by Wollheim (15) The result are given in Tables 1 and 2 and in Fig 1 As shown in Table 1 agglutinating antibodies to *S paratyphi B*

Table 1 *Result of the Widal test*

Age	Agglutination titer			
	S paratyphi B		*S thompson*	
	O	H	O	OH
3 weeks	neg	neg	—	—
5 weeks	neg	neg	—	—
6 weeks	neg	neg	—	—
2 months	—	—	1/40	1/40
3 months	neg	neg	1/5	1/10
3½ months	neg	neg	neg	1/10
4½ months	neg	neg	1/10	neg
6 months	neg	neg	1/40	1/20
9 months	neg	neg	1/20	1/20
2 years	neg	neg	neg	neg

ª The O and H antigens were not completely separated but the H antigen is dominant

O and H antigens could not be demonstrated Agglutination with *S Thompson* O and OH antigens was first tested after 6 weeks of treatment The antibody titer never reached very high levels and 2 years after the infection the Widal test was completely negative

DISCUSSION

The treatment of purulent meningitis in the newborn period involves several problems In the majority of patients the infection is caused by gram negative organisms and the choice of antibiotic(s) may be difficult For salmonella meningitis in the newborn infant ampicillin is recommended and chloramphenicol only when the infecting organism is resistant to ampicillin and then in doses not exceeding 25 mg/kg/day (13) In the case reported here the organism was sensitive to several antibiotics and sulfonamide Ampicillin as a practically nontoxic drug did not cause any discussion It was however deemed desirable to use more than one antibiotic and oxytetracycline was chosen as less toxic than streptomycin and chloramphenicol The risk of dental discoloration was considered of minor importance in this connection and the use of sulfisomidine was thought to be fairly safe as the infant was 9 days old and had no jaundice Judging from the *in vitro* sensitivity

agglutinins by a newborn infant with Salmonella st. paul *J Pediat 68* 780 1966

9 Johansson, S G O & Berg T Immunoglobulin levels in healthy children. *Acta Paediat Scand 56* 572 1967

10 Krentz, M & Elliger E Komplikationen und ungewöhnliche Verlaufsformen der Salmonellosen im Kindesalter *Arch Hyg Bakt 151* 278 1967

11 Krech U & Modde H Die Meningitis salmonellosa. Mikrobiologie und Klinik unter Berücksichtigung eigener Beobachtungen *Helv Paediat Acta 21* 85 1966

12 Mathies A W & Wehrle P F Management of bacterial meningitis in children *Pediat Clin N Amer 15* 185 1968

13 *Med Lett Drugs Ther 10* 50 1968

14 Smith E S Salmonella meningitis in infancy *Am J Dis Child 88* 732 1954

15 Wollheim F Studies on the immunoglobulins in human serum with special reference to γM *Acta Univ Lundensis* section II no 5 Lund 1968

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when the infant had reached an age of three weeks (Table 2) and were then followed up to 2 years When the results are compared with the normal values given in the literature (1 3 9) some questions arise The IgG levels first found in the patient appear to be unusually low and the expected decrease during the first few months of life generally seen in healthy infants could not be observed It has been shown by Berg (5) that in infants with relatively low IgG-levels at birth the decrease is slower than in infants with high levels At the age of 6 weeks the difference between these two groups is small and eventually becomes insignificant The very slow decrease of the patient s IgG levels may therefore be regarded as physiological but it cannot be excluded that the infection also played a certain role Allansmith *et al* (1) have found that in some new borns no drop in the IgG levels can be observed and take this as evidence of an IgG synthesis occurring already during the first weeks of life

It has been shown (2) that 20–30 days after immunization with salmonella antigens the new born at least in many cases develops antibodies of the IgG type The production of IgM antibodies is however the more rapidly occurring and also quantitatively more important immune response It has been observed that newborns produce IgM antibodies both to the flagellar and to the somatic salmonella antigens after artificial immunization as well as after natural infections (6 8) In the present patient the IgM levels did not rise appreciably during the first 6 weeks It might be argued as the level of IgM during the first week of life is not known that the initial value was very low and that the maximal rise had already occurred before the immunoglobulins were first measured at 3 weeks The immune response seems nevertheless to be rather poor with regard to the massive infection and it is tempting to relate this to the relapse which occurred after 1 month s treatment Real evidence of a defective antibody production is however lacking and the immunoglobulin levels found at the age of 2 years are fairly normal even if the IgG values must be regarded as low

The increase in the IgM level at 2 months coincided with an increase in the IgG and IgA levels and can most readily be explained by the three blood transfusions given during the seventh week

SUMMARY

A case of neonatal meningitis caused by *Salmonella Thompson* is presented In spite of adequate antibiotic treatment during 1 month a relapse occurred and treatment had to be continued for 3 months The final outcome was very favourable and at the age of 2 years the patient appeared quite normal During the course of the disease the immunoglobulin levels and the Widal test were followed Evidence of an antibody deficiency syndrome explaining the patient s relapse could not be demonstrated

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REFERENCES

1 Allansmith M McClellan B H Butterworth M & Maloney J R The development of immunoglobulin levels in man *J Pediat 72* 277 1969
2 Altemeier W A & Smith R T Immunologic aspects of resistance in early life *Pediat Clin N Amer 12* 633 1965
3 Bauer U & Freund R Normalwerte von Immunglobulinen im Kindesalter *Mschr Kinderheilk 117* 559 1969
4 Bellanti J A & Jackson A L Characterization of the serum immunoglobulins to the somatic antigen of *S* typhosa in an infant following intrauterine immunization *J Pediat 71* 783 1967
5 Berg T The immunoglobulin development during the first year of life *Acta Paediat Scand 58* 229 1969
6 Gotoff S P & Cochran W D Antibody response to the somatic antigen of Salmonella newport in premature infants *Pediatrics 37* 610 1966
7 Henderson L L Salmonella meningitis *Am J Dis Child 75* 351 1948
8 Hodes H L Zepp H D Ambender E Berger R & Hevizy M Production of O and H

Table 1 *The results of investigations carried out while the patient was receiving fructose in his diet and 8–12 days after the introduction of a fructose free diet*

	After admission	On fructose free diet
Blood		
Haemoglobin (g/100 ml)	8 3	8 3
Platelet count (mm)	71 000	over 250 000
Plasma calcium (mg/100 ml) (normal 8 5–10 5)	8 9	—
Plasma phosphorus (mg/100 ml) (normal 4 0–5 5)	1 8	4 4
Plasma sodium (mEq/l)	177	136
Plasma potassium (mEq/l)	3 3	4 7
Plasma chloride (mEq/l)	102	103
Plasma TCO (mEq/l)	15	21
Liver Function		
Plasma bilirubin (mg/100 ml)	8 4	1 1
Plasma proteins (g/100 ml)	3 9	5 4
Serum transaminases (μM/100 ml serum/hour)		
GOT (normal 10–110)	294	188
GPT (normal 10–110)	270	84
Prothrombin time (control value)	155	120
Partial thromboplastin time (control value)	160	175
Fibrinogen level (control value)	5	—
Urine		
Glucose (mg/100 ml)	0–200	0
Galactose (mg/100 ml)	0–200	0
Fructose (mg/100 ml)	0–200	0

serum GOT level rose from 150 μM/100 ml/hour to 310 μM/100 ml/hour and the GPT from 64 μM/100 ml/hour to 154 μM/100 ml/hour during the first 5 hours after the fructose load Urine passed during the test contained 300 mg fructose/100 ml

These results were considered diagnostic for HFI (1 5)

Amino acid studies

Plasma tyrosine levels around 8 mg/100 ml (normal 1 mg/100 ml) and methionine levels around 7 mg/100 ml (normal 0 3 mg/100 ml) were found while the patient was receiving fructose in his diet The remainder of the plasma amino acid pattern was at the upper limit of normal A heavy generalized amino-aciduria was present at this time and there was also excessive excretion of p hydroxyphenyl acetic and lactic acids in the urine Negative results were obtained when the urine was tested with Phenistix or 2 4 dinitrophenylhydrazine

After 11 days treatment with a fructose free diet the plasma tyrosine had fallen to 2 mg/100 ml but the methionine level remained elevated at 7 mg/100 ml Thirteen days after the introduction of a fructose free diet the urinary amino acid pattern was at the upper limit of normal and phenolic acids could no longer be detected in the urine However excess p-hydroxyphenyllactic acid was detected in urine passed after the fructose tolerance test

DISCUSSION

The clinical and biochemical features of HFI and tyrosinosis may be very similar Both conditions may present during the first months of life with vomiting failure to thrive jaundice enlargement of the liver and ascites Hypophosphataemia hypoglycaemia bleeding disorders and abnormal liver function tests may occur in either condition together with proteinuria aminoaciduria mellituria (glucose galactose or fructose) and renal acidosis (1 3 5 8) The findings in the present case illustrate a further similarity between the two conditions The elevated plasma levels of tyrosine and excess of phenolic acids in the urine which were present while the patient was receiving fructose in his diet suggested a diagnosis of tyrosinosis (3) The correct diagnosis of HFI was only suspected some time after admission when a detailed history was obtained with the assistance of an interpreter

Elevated plasma tyrosine levels have been found in adult subjects with chronic liver disease (6) and the abnormality of tyrosine metabolism noted in the present case was probably secondary to a transient fructose induced disturbance of liver cell function A similar disorder of tyrosine metabolism has been described by Lindemann *et al* (7) in an infant who

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CASE REPORT

ABNORMAL TYROSINE METABOLISM IN HEREDITARY FRUCTOSE INTOLERANCE

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There have been many accounts of hereditary fructose intolerance (HFI) since the condition was recognised by Froesch *et al* in 1957 (2) The clinical features of cases which present in early infancy were reviewed by Levin *et al* (5) who noted the similarity to those seen in tyrosinosis (3) The following report describes an infant with HFI who showed the biochemical features of tyrosinosis while he was receiving fructose in his diet

CASE REPORT

The patient was born after a 32 week pregnancy and weighed 1800 g He was initially fed on a diet which did not contain fructose and made good progress weighing 2500 g at the age of 8 weeks At this time his diet was changed to one containing sucrose and he began to vomit after feeds When he was 11 weeks old he was given larger quantities of sucrose as he had become constipated and during the next 5 weeks his vomiting became more frequent and his condition deteriorated He was admitted to hospital in Athens at the age of 16 weeks with jaundice hepatomegaly and gross ascites which necessitated paracentesis abdominis Elevated serum transaminase levels hypoproteinaemia proteinuria and galactosuria were noted at that time

When admitted to this hospital at the age of 17 weeks he appeared ill and moderately jaundiced His liver extended 7 cm below the costal margin his spleen tip was palpable and he had marked ascites with communicating hydrocoeles The history of recurrent vomiting after sucrose ingestion was not obtained at the time of admission and the patient continued to receive sucrose (15 g/day) in his diet There was no significant change in his condition until 3 weeks after admission when a more complete dietary history was obtained and glucose was substituted for sucrose in his feeds On this fructose free diet he had no further vomiting and within 2 weeks his jaundice had cleared his liver had become smaller and softer and his ascites had resolved completely He was thriving when discharged from hospital at the age of 23 weeks

INVESTIGATIONS

General

The results of investigations carried out before the introduction of a fructose free diet are given in Table 1 The patient had mild anaemia and thrombocytopenia Liver function tests indicated hepato-cellular damage and clotting tests were abnormal Hypophosphataemia and mild acidosis were also found Chromatography of the urine for sugars showed a variable melituria There was no evidence of rickets on X rays of the wrists and chest

Fructose tolerance test

A fructose load (0 5 g/kg) was given by nasogastric tube 16 days after fructose had been excluded from the patient's diet The blood glucose fell from a fasting level of 56 mg/100 ml to 10 mg/100 ml 2 hours after the fructose load The total blood sugar fell from 60 mg/100 ml to 31 mg/100 ml and the plasma phosphate from 6 0 mg/100 ml to 4 0 mg/100 ml during the same period The

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CASE REPORT

SILVER S SYNDROME

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Congenital hemihypertrophy is a well known phenomenon which in less severe cases can hardly b characterized as pathological (10) In more severe cases the hemihypertrophy will be of clinical interest especially if other mal formations occur simultaneously In 1953 Sil ver *et al* (13) described two cases of hemi hypertrophy with several other peculiar abnor malities In 1964 Silver (14) collected a total of 29 cases presenting this entity which is now often termed Silver s syndrome The following features may be observed in this syndrome (14)

Significant asymmetry
Shortness of stature
Small size despite birth at term
Variation in the pattern of sexual development
  Elevated urinary gonadotrophins or
  Early sexual development or
  Premature estrogenization of urethral or vag inal mucosa or
  Markedly retarded bone age in relation to sexual development
Cafe au lait areas of the skin
Unusually short fifth fingers and/or
Increased curve of fifth fingers
Triangular shape of face
Turned-down corners of the mouth
Syndactylism or other abnormalities of the toes

In the following two typical cases of Silver s syndrome are described

CASE REPORTS

Case 1

This boy is the younger of two sibs No miscarriages Family history negative The parents are of normal height Pregnancy reported normal The patient was born at term Birth weight 2,250 g Length 48 cm Normal delivery The right leg was noticed to be longer than the left at the age of 6 weeks

On admission at the age of 3 months to the paediatric ward the patient s weight was 3 500 g height 54 cm There was syndactyly of the third and fourth fingers at the left side hyperflexibility of the finger joints left foot 0 5 cm shorter than the right (Fig 1) left leg was thinner and 1 cm shorter than the right (Fig 2) There was a pronounced tendency to perspiration

At the age of 5 months the boy measured 56 cm i e an increase in height since birth of 8 cm as against the normal increase of 15 cm Weight 3 800 g Furthermore there was a distinct palmar mongoloid crease on both hands The fifth finger was shorter than normal and slightly curved Chromosomes nor mal Serum calcium phosphorus and alkaline phos phatase normal Fractionated serum lipoids normal Serum protein electrophoresis normal Intravenous urography normal X ray of chest and skull normal Ossification centres showed retarded development as compared with his age but identical on both sides Electrocardiogram normal Electroencephalogram nor mal Mental development normal As to the motor development he appeared slightly retarded At the age of 2 years the boy s height was 74 cm and his weight 6 680 g the rate of increase in height is slower than normal and is now distinctly below the 3rd percentile for the normal curve The same applies to his weight

Case 2

This girl is an only child Her mother has formerly had three miscarriages Both parents are tall and stout During the fifth month of pregnancy the mother experienced transitory pains in the abdomen

showed low levels of hepatic fructose 1-phosphate aldolase *post mortem* and Halvorsen & Gjessing (4) subsequently reported elevated plasma tyrosine levels and excessive urinary excretion of phenolic acids in three further infants with HFI It appears that such findings may be common in fructose intolerance and it is clearly important to exclude this diagnosis in any infant with biochemical evidence of tyrosinosis

SUMMARY

Elevated plasma tyrosine and methionine levels, together with excessive urinary excretion of *p*-hydroxyphenyl acetic and lactic acids were found in an infant with hereditary fructose intolerance (HFI) Such findings may be common in HFI and this diagnosis must be considered in any infant with biochemical abnormalities of tyrosine metabolism

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REFERENCES

1 Black J A & Simpson K Fructose intolerance *Brit Med J 4* 138 1967
2 Froesch E R Prader A Labhart A Stuber H W & Wolf H P Die hereditare Fructose intoleranz eine bisher nicht bekannte kongenitale Stoffwechselstorung *Schweiz Med Wschr 87* 1168 1957
3 Halvorsen S Pande H Loken A C & Gjessing L R Tyrosinosis A study of six cases *Arch Dis Childh 41* 238 1966
4 Halvorsen S & Gjessing L Studies on the amino levels in serum and urine of infants and children with metabolic liver diseases *Abstr European Societies for Paediatric Research and Paediatric Gastroenterology* p 43 Sept 1969
5 Levin B Snodgrass G J A I Oberholzer V G Burgess A E & Dobbs R H Fructosaemia observations on seven cases *Amer J Med 45* 826 1968
6 Levine R J & Conn H O Tyrosine metabolism in patients with liver disease *J Clin Invest 46* 2012 1967
7 Lindemann R Gjessing L R Merton B & Halvorsen S Fructosaemia/ Acute tyrosinosis *Lancet I* 891 1969
8 Sass Kortsak A Ficici S Paunier L Kooh S W Fraser D & Jackson S H Secondary metabolic derangements in patients with tyrosinemia *Canad Med Ass J 97* 1079 1967

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Fig 1 Case 1 Short left foot

Delivery was normal The patient was born at term but the birth weight was only 1 320 g and the length 40 cm The placenta was normal The toes on the left foot were missing (Fig 3) The right leg was distinctly thinner and shorter than the left At the age of 9 months the weight was 4 380 g and the height 61 cm Motor development was retarded but mental development normal The right leg was 1 cm shorter than the left (Fig 4) The right arm was al o shorter than the left and the right side of the face slightly flattened There was increased tendency to perspiration

On examination at the age of 18 months normal urinary excretion was found of pituitary gonadotrophins 17 ketosteroids 17 ketogenic steroids and estrogens Chromosomes were normal Urinary excretion of amino acids normal EEG normal X ray of skull and chest normal X ray of the feet showed absence of the second and third phalanges of the fourth and fifth toes on the right On the left side all phalanges were missing

At the age of 18 months the ossification centres were found to be retarded corresponding to the age of 3–6 months but identical on both sides At the age of 15 months distinct estrogenization of the vaginal mucosa was demonstrated

Since then the patient has been followed up in our out patient clinic and has presented acceleration of the weight increase and almost normal rate of increase in height Hence at the age of 2 years the height is 75 cm and the weight 7 500 g

DISCUSSION

The first four symptoms are sometimes described as the major symptoms, the others as minor symptoms According to Reister & Scherz (9) three major symptoms will be sufficient to establish the diagnosis and hence both our patients fulfil the criteria for the diagnosis of Silver s syndrome

Occasionally the asymmetry is described as hemihypertrophy but as stated by Ferrier et al (3) the term asymmetry is to be preferred since it might be difficult to decide whether a genuine hemihypertrophy is present or whether it is a question of a corresponding hemihypotrophy

The asymmetry can vary greatly from mild and localized cases to severe total asymmetry (14) Mild cases might escape recognition at birth but will become evident during growth The asymmetry can be unilateral or crossed (15) Since the asymmetry might occasionally be very slight there is nothing to prevent us



Fig 2 Case 1 The left leg is shorter and thinner than the right

from regarding Russel's intrauterine dwarfism (11) as a variant of Silver's syndrome. However, it should be pointed out that Szalay (16) believes the asymmetry to be of decisive importance for establishing the diagnosis of Silver's syndrome. The asymmetry in these cases cannot be distinguished from other types of asymmetry. However, the well known relationship between hemihypertrophy and Wilm's tumour (5) has not been observed in Silver's syndrome, but this might be due to the fact that the number of cases of the syndrome hitherto described is very limited.

In Silver's syndrome the birth weight is always low in spite of normal duration of pregnancy. According to Black (1) the birth weight must be lower than 2050 g before the term 'low birth weight' can be applied. In our Case 1 the birth weight is slightly above this limit but definitely below normal birth weight and as in the case described by Hook & Yunis (7) the increase in weight during the neonatal period was clearly reduced to below the 3rd percentile. Patients with Silver's syndrome very seldom present birth weights as low as that of our patient No. 2 (1 320 g) but in contrast with patient No. 1 the weight gain increased in this patient although at the age of 2 years she had not yet attained the 3rd percentile. In addition to the birth weight, also the length at birth is retarded (14). However, our patient No. 1 was of normal length at birth, but later he



Fig. 4. Case 2. The right leg is shorter and thinner than the left.

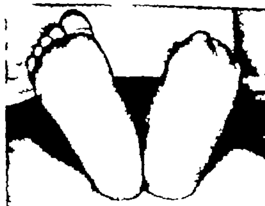


Fig. 3. Case . Absence of the toes on the left foot.

presented a distinctly reduced increase in length and is now below the 3rd percentile.

The retarded birth length follows the patient throughout the growth period and remains usually below the 3rd percentile for normal subjects, as opposed to premature infants (1). Generally the growth curve runs approximately parallel with the normal curve (14).

The development of the ossification centres can be normal (13), but in the majority of cases it is retarded (2, 15) as compared with the age of the patient, although in most cases it corresponds to the growth in length. Occasionally the development of the ossification centres can be more retarded on the affected than on the unaffected side (3, 12). Moseley *et al.* (8) described two patients with accelerat



Fig 1 Case 1 *Short left foot*

Delivery was normal The patient was born at term but the birth weight was only 1 320 g and the length 40 cm The placenta was normal The toes on the left foot were missing (Fig 3) The right leg was distinctly thinner and shorter than the left At the age of 9 months the weight was 4 380 g and the height 61 cm Motor development was retarded but mental development normal The right leg was 1 cm shorter than the left (Fig 4) The right arm was also shorter than the left and the right side of the face slightly flattened There was increased tendency to perspiration

On examination at the age of 18 months normal urinary excretion was found of pituitary gonadotrophins 17 ketosteroids 17 ketogenic steroids and estrogens Chromosomes were normal Urinary excretion of amino acids normal EEG normal X ray of skull and chest normal X ray of the feet showed absence of the second and third phalanges of the fourth and fifth toes on the right On the left side all phalanges were missing

At the age of 18 months the ossification centres were found to be retarded corresponding to the age of 3-6 months but identical on both sides At the age of 15 months distinct estrogenization of the vaginal mucosa was demonstrated

Since then the patient has been followed up in our out patient clinic and has presented acceleration of the weight increase and almost normal rate of increase in height Hence at the age of 2 years the height is 75 cm and the weight 7 500 g

DISCUSSION

The first four symptoms are sometimes described as the major symptoms the others as minor symptoms According to Reister & Scherz (9) *three major symptoms* will be sufficient to establish the diagnosis and hence both our patients fulfil the criteria for the diagnosis of *Silver s syndrome*

Occasionally the asymmetry is described as hemihypertrophy but, as stated by Ferrier et al (3) the term asymmetry is to be preferred, since it might be difficult to decide whether a genuine hemihypertrophy is present, or whether it is a question of a corresponding hemihypotrophy

The asymmetry can vary greatly from mild and localized cases to severe total asymmetry (14) Mild cases might escape recognition at birth but will become evident during growth The asymmetry can be unilateral or crossed (15) Since the asymmetry might occasionally be very slight there is nothing to prevent us



Fig 2 Case 1 The left leg is shorter and thinner than the right

11 Russell A A syndrome of intra-uterine dwarfism recognizable at birth with cranio-facial dysostosis disproportionately short arms and other anomalies *Proc Roy Soc Med 47* 1040 1954
12 Schumacher G & Niederhoff H Das Syndrom nach Russell und Silver *Helv Paediat Acta 22* 404 1967
13 Silver H K Kiyasu W George J & Deamer W C Syndrome of congenital hemihypertrophy shortness of stature and elevated urinary gonadotrophins *Pediatrics 12* 368 1953
14 Silver H K Asymmetry short stature and variations in sexual development *Amer J Dis Child 107* 495 1964
15 Szool S & Cohen P Silver's syndrome *Amer J Dis Child 105* 199 1963
16 Szalay G C Intrauterine growth retardation versus Silver's syndrome *J Pediat 64* 234 1964
17 Tanner J M & Ham T J Low birthweight dwarfism with asymmetry (Silver's syndrome) Treatment with human growth hormone *Arch Dis Childh 44* 231 1969

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ed development of the ossification centres but these two patients were 11 and 15 years old, respectively and possibly this might explain the accelerated bone development Experience with respect to the course during adolescence in patients with Silver s syndrome is still too limited to decide whether in all patients the development of the ossification centres will accelerate during adolescence According to Sil ver (14) early sexual development can be de monstrated in about one third of the patients but in spite of this feature no acceleration in the rate of increase in length occurs The youngest patient hitherto observed presenting increased urinary excretion of gonadotrophins was 17 months old (2) In our Case 2 the ex cretion of gonadotrophins was normal but vaginal secretion showed estrogenization as early as the age of 15 months Ferrier et al (4) described estrogenization of vaginal smears in two children without asymmetry who in other respects fulfilled the criteria for the diag nosis of Silver s syndrome

The incidence of minor symptoms varies considerably and similar changes are frequently observed in the ordinary types of hemihyper trophy (10)

Mental development can be retarded in Sil ver s syndrome (3) but is normal in most cases Both our patients presented increased tendency to perspiration but as in the case described by Girard & Kaufmann (6) we have not been able to detect any metabolic cause of this phenomenon

The etiology is completely unknown and may be multifarious Chromosome studies in most cases reveal normal conditions However Hook & Yunis (7) described one case with 18 trisomy and Ferrier *et al* (3) one case with diploid–triploid mosaicism Familial occurrence or sex differences have not been demonstrated (14) Growth hormone studies have been made in a few cases only (6 17) and in one case only was reduction found (17)

There is no possibility of treating the asymmetry Only in very isolated cases have attempts been made to treat the growth retardation with growth hormone (17) During this treatment the growth in length increased considerably but the normal level was not reached in spite of treatment for 1 to 3 years In patients with normal production of growth hormone this effect might indicate that the patient s own growth hormone is not normal or that the patient does not react adequately to a normal production of growth hormone However ex perience with growth hormone treatment in Sil ver's syndrome is too sparse as yet to permit an evaluation of the effect

SUMMARY

A description is given of a boy and a girl with asymmetry shortness of stature and low birth weight despite being born at term The girl showed estrogenization of the vaginal mucosa at the age of 15 months No chromosomal ab normalities were demonstrated

REFERENCES

1 Black J Low birth weight dwarfism *Arch Dis Childh 36* 633 1961
2 Cerri J F J Vanucci R C Grossman H & New M Elevated serum gonadotrophins in Silver s syndrome *Amer J Dis Child 114* 651 1967
3 Ferrier P Ferrier S Stalder G Buhler E Bamatter F & Klein D Congenital asymmetry associated with diploid triploid mosaicism and large satellites *Lancet I* 80 1964
4 Ferrier P Shepard T H & Smith E K Growth disturbances and values for hormone ex cretion in various forms of precocious sexual development *Pediatrics 28* 258 1961
5 Fraumeni J F Jr Geiser C F & Manning M D Wilm s tumor and congenital hemihyper trophy *Pediatrics 40* 886 1967
6 Girard J & Kaufmann H J Der Russel Zwerg *Mschr Kinderheilk 113* 696 1965
7 Hook E B & Yunis J J Congenital asym metry associated with trisomy 18 mosaicism *Amer J Dis Child 110* 551 1965
8 Moseley J E Moloshok R E & Freiberger R H The Silver syndrome Congenital asym metry short stature and variations in sexual development *Amer J Roentgen 97* 74 1966
9 Reister H C & Scherz R G Silver syndrome *Amer J Dis Child 107* 410 1964
10 Ringrose R E Jabbour J T & Keele D K Hemihypertrophy *Pediatrics 36* 434 1965

the summer of 1968 This shows that poliomyelitis has in fact not been eradicated

The incidence of polio in Finland after the intense vaccination with inactivated poliovaccein in the years 1960–62 showed a sudden drop Only 2 virologically diagnosed cases have occurred since both had contracted the infection abroad In 6 other suspected cases the diagnosis could not be confirmed virologically

In spite of this satisfactory epidemiological situation a survey of the present vaccination program and the immunity status of the population shows that some risk of a decrease in the immunity of the population is at hand

To avoid incidents like the one in Poland it is suggested that the immunity status of the population be surveyed at intervals and that search for enteroviruses from sewage should be continued

Vaccination of children and revaccination of young women should be intensified The young men already receive a booster in military service Finally live vaccine should be available for emergency

Discussion

R Tammilehto Live poliomyelitis vaccine is now in use in most countries but *in Finland* and Sweden inactivated vaccine is *still utilized* Vaccination with live vaccine would probably result in 4–5 cases of poliomyelitis annually This risk would be worth taking only if the annual number of poliomyelitis cases would exceed this number

P Halonen *Current position of measles vaccines*

In general Finnish pediatricians have assumed a very cautious attitude to measles vaccinations This may be partly caused by the mild nature of measles in Finland and the great decline in number of deaths caused by this disease over a 30-year period In 1939 412 deaths were reported out of 55 000 measles cases (7 deaths/1 000 notified cases) During last 5 years the mean annual incidence has been about 20 000 and the number of deaths 3 (0 15 death/1 000 notified cases) The last figure is almost identical with measles mortality in England (0 2 death 1 000 notified cases) However the precise incidence of complications especially of postinfectious encephalitis and of late neurological disorders such as SSPE and multiple sclerosis (?) is not known Another reason for cautious attitude is the possibility that vaccinations will postpone the disease 10 to 20 years resulting in very severe forms of measles in adults This problem however can be well avoided by revaccinations if necessary

The experience with inactivated measles vaccines has been disappointing the potency being low In addition children immunized with inactivated vaccines and later exposed to natural measles have developed measles with atypical skin reactions *and* severe pneumonia indicating hypersensitivity to measles virus

With live further attenuated measles virus vaccines a huge experience has accumulated Approximately 40 million doses of vaccines have been used in the USA and other countries The protective efficacy has been clearly demonstrated by the dramatic decline in measles *in the* USA the figures for epidemiological years 1967–68 and 1968–69 being 10 or less of the prevaccination average The serological tests indicate that immunity should last long if not as long as after disease HI antibody response in vaccinees resembles that of measles patients though after vaccination the titers *decrease slightly* faster during 6–7 years than after natural disease

The reactions caused by live measles vaccines depend on the attenuation level No gamma globulin is given with the more attenuated strains A higher fever and a modified rash occurs only occasionally with these vaccines Interest has recently focused in more severe illness such as encephalitis associated with measles vaccinations In the USA the rate of neurological manifestations has been approximately 1 out of 200 000 vaccinees compared to 1 400 or 1 1000 after natural measles and in no

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

THE FINNISH PAEDIATRIC SOCIETY

Meeting Oct 18 1969

(together with the Finnish Society for the study of Infectious Diseases)

K Penttinen *Prophylaxis of epidemic influenza by vaccinations*

In spite of vaccines available against epidemic influenza the disease shows the same epidemic patterns as before In Scandinavian countries the number of doses of vaccines used in the beginning of the Hong Kong epidemic were the following Sweden 1 100 000 Finland 200 000 Denmark and Norway 100 000 The ineffectiveness of the prophylactic vaccinations is partly due to the nonavailability of vaccines when a new strain appears and partly to the low efficiency rate of vaccines available At best the protection rate of influenza vaccines is 2–3 and often less Monovalent Hong Kong strain vaccine in Finland only gave a 20–30% protection (L Haapanen unpublished observation) The data available from other countries are equally poor The influenza vaccines have the lowest efficiency of all vaccines in use They are not recommended for general unrestricted use but only for medical risk groups according to the judgement of a physician In such a group the effectiveness of vaccination (0–50 %) is not critical everything possible must be done Another group which might be considered is medical personnel who take care of severely ill patients

The reasons for the low efficiency are now becoming clear Influenza is a surface infection without a viremic stage Antibodies in serum are of minor importance as compared with local IgA The decision to use or not to use influenza vaccines is not much influenced by the complications of the vaccination Theoretically there are many unfavorable possibilities, but the use of millions of doses without reported serious complications has tranquilized the manufacturers and vaccinators The use of vaccines with only one or two components seems reasonable because of the possible competition between the similar antigens The best vaccination schedules should be worked out A new approach is the use of purifed particle vaccines which probably can be injected or inhaled

DISCUSSION

Liisa Haapanen The staff of the Aurora hospital was vaccinated in 1968 with a single dose of monovalent Hong Kong strain vaccine Comparison with a control group showed no statistically proved protection Neither was the prophylactic use of Amantadine effective

N Oker Blom In medical students a 60–70% protection was obtained with Amantadine

N Oker Blom *Vaccination against polio*

Poliomyelitis has practically disappeared from most of the countries in the Western hemisphere thanks to intense vaccination programs In Poland about 600 cases of poliomyelitis occurred in the Poznan region within a few months in

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PAEDIATRIC GASTROENTEROLOGY

Meeting in Interlaken September 15 1969

J Schmitz & J Rey (Paris) *Characteristics of glycyl L leucine and glycyl L valine dipeptidase activities in the human intestine*

The hydrolysis of two very similar substrates (glycyl L leucine and glycyl L valine) by an homogenate of human intestinal mucosa was studied to prove the possibility of the hydrolysis of several peptides by one enzyme pH optimum (7 7 and 8 0) heat inactivation temperature (50 and 53 C) and apparent Km (0 03 M/1) of the two activities using Josephson & Lindberg s assay method were appraised

The very similar results suggest that both activities take place at one site However using a Technicon Autoanalyzer with a short column (30 cm filled with type XX 8 60-0 Phoenix resin) it was shown that there is no competitive inhibition between the two substrates since glycyl L leucine reduces the activity of glycyl L valine dipeptidase to half without modifying its apparent Km This unexpected result was corroborated by the lack of influence of a mixture of glycine and leucine on the hydrolysis of glycyl L valine implying that this non-competitive inhibition is not related to the liberation of amino-acids Hence it was of interest to investigate the possible influence on glycyl L valine dipeptidase activity of a dipeptide very probably not hydrolysed by the same enzyme as glycyl L valine and glycyl L leucine For this purpose alanyl L proline was chosen and it was found that alanyl L proline too reduces although less than glycyl L leucine the hydrolysis of glycyl L valine without modification of the apparent Km

Further studies are necessary to clarify and ascertain these first results anyhow the "one site" hypothesis for these two activities must be reconsidered

P Kuitunen K Krohn & J K Visakorpi (Helsinki) *Gastric mucosal changes and antibodies to intrinsic factor and to the parietal cells in some endocrine disorders in childhood*

The occurrence of gastric atrophy and pernicious anaemia in hypoparathyroidism is a well known syndrome especially in adults Pernicious anaemia combined with gastric atrophy may occur in some other endocrine disorders such as thyroid diseases and Addison s disease

The gastric mucosa its function the absorption of vit B_{12} and the antibodies to intrinsic factor (according to Ardeman and Chanarin) and to parietal cells (indirect immunofluorescence) were investigated in 9 hypoparathyroid children ($3\,^{6}/_{12}$ – $16\,^{6}/_{12}$ years) and 20 children (9–$18\,^{1}/_{12}$ years) with autoimmune thyroiditis

Three of 9 children with idiopathic hypoparathyroidism had achlorhydria Two of them also had pernicious anaemia gastric atrophy and antibodies to parietal cells and to intrinsic factor These patients also had steatorrhea despite a normal jejunal mucosa and one of them had Addison s disease as well The third achlorhydric patient had a borderline Schilling test and partial villous atrophy of the jejunal mucosa but a normal gastric mucosa In the other hypoparathyroid patients gastric studies were normal except in two who were hypo-

case has a relationship between the encephalitis or death and the vaccination been definitely proved This rather small indirect evidence of more severe complications occurring after natural measles has shifted the selection of vaccine strains to more attenuated ones

There is no further laboratory clinical or epidemiological data to be expected on which the decision to use a vaccine in Finland could be based Commencement of vaccination in one or two health districts with gradual increase to cover the whole country seems to be a reasonable approach in our country The practical steps depend now on the activity of health officers and on the interest of pediatricians rather than on virologists

Discussion

K *Penttinen* Is the frequence of SSPE higher after natural measles than after vaccination against measles?

P *Halonen* Nobody knows!

M Valle *Vaccination against smallpox*

In spite of world wide vaccination programmes about 80 000 cases of smallpox are still recorded annually During the past 20 years there have been about 80 outbreaks of the disease in the smallpox free countries The great increase in air travel has increased the risk that smallpox could be brought into our country It is thus necessary continuously to control the vaccination certificates of passengers arriving from smallpox countries and from outbreak areas Two features of smallpox outbreaks in smallpox free countries should be emphasized First the primary case is most probably modified which means that the diagnosis may be difficult especially if the possibility of smallpox is not kept in mind Secondly smallpox spreads mainly by close contact and the risk of transmission is very high in hospitals Thus hospital personnel should be properly vaccinated annually

As vaccination complications occur more often in patients who have certain allergic disease are on glucocorticoid therapy or are pregnant these should not be vaccinated When these people travel to another smallpox free country vaccination against smallpox should not be required If such individuals have to be vaccinated during smallpox outbreaks or when travelling to endemic areas antivaccinial gamma globulin should be given simultaneously To lower the risk of postvaccination encephalitis primary vaccination is recommended to be performed at the age of about one year

In Finland a liquid calf lymph vaccine is used The preparation of freeze dried smallpox vaccine which will be donated to WHO has been started This will also be available on request for vaccinations in our country The multiple puncture (with a bifurcated needle) or the multiple pressure techniques are the vaccination methods recommended in Finland

It is emphasized that the vaccination reaction should be observed and all complications reported

Meeting Dec 13 1969

Juvenile use of narcotics

Panel Discussion

Participants N Hallman M D chairman J Seise municipal attorney J Idanpaan Heikkila M D Pirkko Idanpaan Heikkila M D E Vario M D and R Jaakkola M A

E I Wallgren

technique was used using as substrate an inter esterified mixture of natural oils and trimyristine in order to obtain as closely as possible the same percentage of six fatty acids lauric myristic palmitic stearic oleic and linoleic distributed at random on the glycerol molecules The triglycerides were isolated on a silicic acid column after heat inactivation of the lipase lipid extraction by a system of ether heptane ethanol and water and saponification of free fatty acids

The relative composition of recovered triglycerides is the same as that of ingested oil for fatty acids of more than 12 carbon atoms (index of recovery ≈1) the percentage of lauric acid on the other hand is significantly diminished (index of recovery 0 5±0 2) The results confirm that the rate of hydrolysis of fatty acids of 14–18 carbon atoms is independent of the chain length and the degree of saturation This does however no imply that pancreatic lipase has a particular affinity for lauric acid the findings may also be explained by the fact that the fatty acid is more soluble in the aqueous phase and that isomerisation from positions β to α of the glycerol molecule is more rapid when lauric acid is compared with longer fatty acids

W Plenert U Spahn & E Petrich (Jena) *Serum lipids in the newborn and young infant—Influence of nutrition*

There is a typical serum lipid pattern of the newborn immediately after birth which is roughly characterized by a very low level of all lipid fractions and a remarkably small quantity of linoleic acid Already in the course of the first few days a rapid shift of the serum lipid pattern can be observed and it takes only a short time till the serum lipids level up to the values found in later infancy These changes are dependent on the regime of nutrition especially on the quantity of dietary fat Our own recent studies were concentrated on the questions are other factors than the dietary fat intake correlated with the postnatal changes of serum cholesterol and does the intake of food cholesterol influence the value of serum cholesterol Though the total cholesterol increased in the first days of life the cholesterol linolate was found to decrease while the other ester fractions especially oleate and the esters of tri and tetraenes showed a marked elevation There was no evidence that the percentage of esterified cholesterol was affected essentially by immaturity at birth or by hyperbilirubinemia During nutrition with human milk an immediate higher rate of cholesterol linolate ester synthesis in the second and third week of life can be expected while the concentrations of tri and tetraene esters decrease Likewise beyond the postnatal period no influence of body weight degree of maturation at birth or different course of postnatal period could be registered Our findings support the conclusion that alimentary factors prevail in the development of serum lipid patterns from birth to later infancy

There is another difference in the level of serum cholesterol correlated to the regime of nutrition In accordance with other investigators there was a considerable rise of total serum cholesterol in infants fed human milk In contrast total serum cholesterol was significantly lower in infants fed modified cow's milk These adapted formulas are characterized by replacement of butterfat by vegetable oils rich in polyenoic acids The lower levels of serum cholesterol in infants fed adapted formulas have been attributed to the depressing effect on serum cholesterol of an high intake of polyenoic acids This hypothesis has not been proved and it lacks some sound evidence as the intake of polyenoic acids with human milk is far from low Recently it has been shown in adults that the intake of dietary cholesterol may influence the serum cholesterol level significantly Our own findings suggest that the intake of dietary cholesterol is relatively high in infants fed human milk and considerably lower in infants fed adapted formulas filled with vegetable oils

chlorhydric Elevated immunoglobulins was mostly due to raised IgG The patients with idiopathic hypoparathyroidism seemed first to suffer from hypoparathyroidism conjoined with Addison s disease and later from gastric atrophy conjoined with pernicious anaemia

In autoimmune thyroiditis achlorhydria was found in 3 patients Two of these had gastric atrophy and one had atrophic gastritis grade 1–2 Five more patients had superficial or interstitial gastritis but no abnormalities in gastric function Pernicious anaemia was found in only 1 case with gastric atrophy Antibodies to parietal cells were found in 5 of 8 patients with and in 3 of 11 patients without gastric mucosal changes In autoimmune thyroiditis concomitant disorders were found diabetes mellitus in 3 malabsorption syndrome with jejunal villous atrophy in 3 lack of immunoglobin A in 2 and rheumatoid arthritis combined with nephropathia in 1 patient

The results of this study demonstrate that even in childhood pernicious anaemia occurs rather frequently in conjunction with endocrinopathies Our findings indicate an immunologic mechanism which perhaps continues for several years

G Zoppi (Ferrara) D H Shmerling D Gaburro & A Prader (Zurich) *The electrolyte and protein contents and outputs in duodenal juice after pancreozymin and secretin stimulation in normal children and in patients with cystic fibrosis*

The duodenal contents and the secretion of Na^+ K^+ Ca^{++} Mg^{++} $HCO_3^=$ and total protein were studied after pancreozymin and secretin stimulation in 12 healthy control children and in 5 patients with cystic fibrosis of the pancreas (CF) Duodenal contents were collected through a double balloon triple lumen duodenal tube thus avoiding contamination by gastric juice and distal losses

In the controls the following secretion rates were found (means ±1 SD mg/kg/50 min after i v stimulation) Na^+ 13 9±6 4 K^+ 0 8±0 3 Ca^{++} 0 12±0 05 Mg^{++} 0 05±0 01 $HCO_3^=$ 15±7 protein 15 4±5 5 volume 3 9±1 6 ml/kg/50 min

In CF patients decreased secretion rates were found Na^+ 2 5±0 3 K^+ 0 2±0 07 Ca^{++} 0 04±0 02 Mg^{++} 0 03±0 02 $HCO_3^=$ 1 4±1 5 protein 9 7±8 6 volume 0 8±0 4 ml/kg/50 min The ratio Na^+ to K^+ secretion were found to be 17 4 in the controls and 12 5 in CF patients and the ratio Ca^{++}/protein 0 008 in the controls and 0 004 in CF

Secretion velocity expressed in µg/kg/min, of the electrolytes and of protein was analyzed separately for the post pancreozymin and the post secretin periods i e for the acinar and the tubular secretion phases In normal controls the secretion velocities of Na^+ K Mg^{++} and $HCO_3^=$ were higher after secretin stimulation whereas those of Ca^{++} and protein were higher after pancreozymin stimulation In CF patients Na^+ K^+ Mg^{++} and $HCO_3^=$ secretion velocities after secretion were somewhat lower than after pancreozymin and the values for Ca^+ and protein did not show any significant change Compared to the values in normals the secretion velocities of Na K Mg + and $HCO_3^=$ in CF patients are more depressed after secretin than after pancreozymin stimulation whereas Ca^+ and protein were more affected during the latter phase The authors discuss the possible role of a disturbance of the tubular secretion of ions and of the acinar production of Ca protein complexes in the genesis of symptoms in this disease

C Ricour & J Rey (Paris) *Hydrolysis rates of long chain triglycerides Study in vivo*

The fatty acid composition of triglycerides remaining in the intestinal lumen during fat digestion was analyzed to study the relation between the rates of hydrolysis of triglycerides and the chain length and the degree of saturation of the fatty acids After intubation of first jejunal loop with a single lumen tube the test meal

persisted in 3 patients and in Case 5 the intestinal mucosa and the disaccharides returned to normal when tested 3 months after the initiation of therapy. Clinical improvement in all cases was striking. We believe that this mode of therapy was life saving in these 5 infants.

Jos (Paris): *Immunohistochemical study of the intestinal mucosa in children*

An immunohistochemical study of the intestinal mucosa has been performed in 37 children comprising controls (9), coeliac disease (17), cow's milk intolerance (7), intestinal lymphangiectasia (1) and gammaglobulin deficiency (1 alymphocytosis, 1 Bruton disease and 1 "acquired" hypogammaglobulinemia).

Duodeno-jejunal biopsies were obtained with the Crosby capsule and were immediately frozen. Sections 6 μ thick were cut in a cryostat at −22 C and after fixation in absolute methanol they were incubated with fluoresceinated antisera. The slides were examined and photographed under ultraviolet light using a dark field condenser. The specificity of the antisera (anti IgA, IgM and IgG immunoglobulin) was checked by immunoelectrophoresis and by double immunodiffusion in Ouchterlony plates.

The results demonstrate that the lamina propria of intestinal mucosa in normal children contains, as in adults, large numbers of IgA type plasma cells. The amount of the IgM and especially the IgG type cells is much smaller.

In untreated coeliac disease the number of fluorescent cells using IgA immunoglobulin antiserum is always increased and inconstantly so using anti IgM. This cellular hyperplasia disappears when gluten is omitted from the diet for several months. Cellular hyperplasia reappears a few weeks after reintroducing gluten in the diet of the patients. Corresponding changes are observed in cow's milk intolerance.

In primary antibody deficiency states (Bruton disease, "acquired" hypogammaglobulinemia, alymphocytosis) no immunoglobulin-containing cell is found in the lamina propria of intestinal mucosa. In secondary types such as lymphangiectasia or malabsorption syndromes the number and the distribution of the IgA-containing cells are normal or increased. No case with malabsorption or mucosal alterations and isolated IgA-deficiency was observed.

These immunofluorescent studies appear useful to investigate the changes in immunoglobulin synthesis associated with gastrointestinal disorders and to obtain accurate information about the nature of the cellular hyperplasia of the lamina propria seen in malabsorption syndromes.

S. Nordio & G. Moscatelli (Trieste): *Studies on intestinal mucosa and PHA stimulated lymphocytes in malabsorption syndrome and immunologic deficiencies of children*

The authors take once more into consideration the results of a research on a few enzyme activities of *intestinal* mucosa and the PHA responsiveness of *lymphocytes*.

A statistically significant increase of intestinal beta-glucuronidase is chiefly emphasized in malabsorption syndromes. It is the *first* time *that an increase instead of a decrease of an enzyme is observed in this* syndrome. Since beta glucuronidase in a lysosomal enzyme the *role of lysosomes in* the pathogenesis of malabsorption syndrome *might* be taken into consideration. In fact an increase of lysosome like bodies *of epithelial* cells of intestinal mucosa was *demonstrated by Shiner* and the authors. But in *interpreting* hyperactivity of beta glucuronidase immuno-competent lymphoid cells of *intestinal* mucosa cannot be forgotten. PHA stimulation of in vitro cultivated lymphocytes *provokes* in control subjects not only blast transformation but an increase of beta glucu*ronidase too*. In 1 case only of malabsorption syndrome was blast transformation reduced while in 2 other cases betaglucuronidase of PHA *stimulated* lymphocytes increased in a lesser degree. Similar results were obtained in *a case of a* gamma globulinemia and in a case

D Nussle J Frei C Bozic & E Gautier (Lausanne) *Proximal and distal lesions in malabsorptive states A morphological enzymatic and functional study*

Twenty children with coeliac disease aged 6 months – 15 years were investigated

In order to assess the extension of the lesions a proximal and a distal jejunal biopsy was obtained, using the Crosby capsule The distal biopsy was taken 120–150 cm below the ligament of Treitz A small mercury bag was used to propel the capsule and metoclopramide was given in repeated doses The mucosal specimens were examined under a dissecting microscope as well as histologically Enzyme activities of lactase sucrase maltase and alcaline phosphatase were measured Furthermore, balance studies and loading tests were performed in the patients

Similar to the great variation in the severity of the clinical manifestations of coeliac disease we found great variations between patients in the extent of the morphological lesions and in the impairment of the enzyme activities

A close relationship between the extension of the morphological lesion and the degree of the malabsorption did not exist but steatorrhea was regularly found if the distal mucosa showed a partial or subtotal atrophy

A better correlation was found between the overall lactase activity as estimated from the sum of proximal and distal lactase activities and the tolerance to lactose in these patients Intolerance to lactose occurs only if the lactase activity at both sites is markedly decreased

There is no fixed relationship between the morphology and the enzyme activities especially not in the presence of moderate morphological alterations of the distal mucosa

H Shwachman R M Filler & K T Khaw (Boston Mass) *A new method of treating malnourished infants with severe chronic diarrhoea*

A newly described technique of total parenteral nutrition devised by Wilmore & Dudrick has been used in our institution over the past year with some modifications A detailed description of the method is presented in the *New England Journal of Medicine 281* 589–594 1969 The details of the preparation and composition of the infusate its placement and proper care and the routine clinical measurements on some of the patients studied will be found in the article mentioned

This method has been used in the treatmen of 5 infants with severe malnutrition secondar to chronic diarrhoea who failed to respond t the usual medical measures including I V therapy Four of these patients were treated a other hospitals without success prior to the transfer to our hospital These infants had a similar history with onset of diarrhoea and vomiting shortly after birth inability to tolerat formula (and many were tried) no fever excep in 1 patients with a diagnosis of otitis media Many laboratory tests were performed all wit a variety of negative results The following tabl lists the age the life line was instituted the duration of this therapy and the weight of the infant at the onset and at the end of the parenteral therapy

	Age at start (days)	Duration (days)	Total administratio weight kg start	en
Patient 1	56	44	2 9	38
2	65	39	3 3	45
3	150	52 +	4 0	53
4	140	51	3 8	53
5	77	30	3 6	48

Oral feedings were instituted gradually firs with clear solutions and the initial formul given was a special preparation prepared b Mead Johnson Co (3200AD) (Dr Herber Sarett) which was composed of glucos M C T casein hydrolysate starch plus vita mins and minerals

Serial intestinal biopsies were performed in infants At the beginning of the special therap on 3 infants and on several subsequent occa sions Histologic changes were noted in al cases with Grade 2 to 3 villous atrophy Ther was also a reduction in activity of lactase mal tase and sucrase This secondary deficiency

Alexandra Hospital for Children have been studied The following descriptive terms have been used "Fingers" described narrow villi with parallel sides Broader villi were divided into narrow "leaves" "tongues" and thin ridges depending on their width Short thick ridges described a mucosa where villi were replaced by thickened ridges sometimes having a convoluted appearance

There were 46 neonates 39 children between 1 month and 1 year and 21 over 1 year of age Fifty nine of the 106 children had evidence of gastroenterological disease

Thin ridges leaves and tongues were most often seen in the duodenum and proximal jejunum of the non gastroenterological group with fingers and narrow leaves in the ileum The gastroenterological group often had short thick ridges in the proximal gut These changes were most marked on the tops of the plicae circulares where at times the mucosa was almost flat Tongues and narrow leaves were more frequent than fingers in the ileum of these children However the chief differences between the two groups occurred in the proximal small bowel and this was the region of the severest abnormality although in some the whole small intestine was involved Apart from a higher percentage of neonates having finger like villi in the ileum there did not appear to be a significant difference of appearances in relation to age

M Gracey V Burke & Ch M Anderson (Birmingham) *Malabsorption of carbohydrate associated with abnormal intestinal flora*

In the blind loop syndrome steatorrhea and deconjugation of bile salts are related to the presence of abnormal bacterial flora within the small bowel Disturbances of fat and vitamin B absorption and of protein metabolism have previously been demonstrated in this syndrome However carbohydrate malabsorption occurring in the presence of bacterial overgrowth in the small bowel has not been generally recognized

We have demonstrated impairment of absorption of carbohydrate in 8 babies with abnormal intestinal bacterial flora All patients presented with severe watery diarrhoea and dehydration following the ingestion of glucose, galactose or fructose

The patients are considered in two clinical groups

1 Four newborns presenting following surgery for the relief of small bowel obstruction

2 Four babies presenting following an illness resembling gastroenteritis

Carbohydrate malabsorption was determined semi quantitatively by using the Clinitest method to estimate concentration of stool sugar

In all patients there was an abnormality of intestinal flora (mainly overgrowth of *E coli*) which paralleled the severity of symptoms Unconjugated bile salts were present in samples of intestinal juice from all patients while monosaccharide malabsorption was present Small bowel biopsy in the nonsurgical group showed normal histology and disaccharidase activity In the surgical group dilatation of the proximal intestinal segment results in stasis however barium studies showed no anatomical abnormalities in the non surgical patient

The mechanism of production of this form of carbohydrate malabsorption which involves the active transport of glucose and galactose and the more passive transport of fructose is not presently known but is under study

C Borrone F Dagna Bricarelli L Massimo A Fossati Guglielmoni & P Durand (Genoa) *Lymphocyte transformation in milk allergy*

Blast transformation of lymphocytes from peripheral blood in the presence of milk proteins proved the clinical diagnosis of milk allergy in 2 children 1 of them suffering from hyposideremic anaemia from chronic intestinal bleeding the other one from urticaria and collapses (Borrone *et al* *Min Ped* *21* 1070 1969)

with severe hypogamma globulinemia In these subjects beta glucuronidase was also reduced in intestinal mucosa

B Strandvik R Zetterstrom & A Norman (Stockholm) *Malabsorption during the course of cholestasis of infancy*

The results of studies on 6 infants with cholestasis were presented Two infants with intrahepatic cholestasis were found to have an impairment of fat absorption as marked as in 2 infants with extrahepatic biliary atresia The degree of steatorrhea was found to be correlated to the impairment of the excretion of bile acids into the intestine

Follow up studies of 4 infants with intrahepatic cholestasis demonstrated that intestinal bile acid excretion and consequently the absorption of triglycerides and of vitamin A were impaired as long as jaundice persisted Bile acid excretion into the intestine as well as the absorption of fat and vitamin A were found to normalize in the 2 patients in whom hyperbilirubinemia disappeared

J H van de Kamer (Zeist) & H A Weijers (Utrecht) *Lactic acid in faeces*

The estimation of the lactic acid content of faeces is of use in the diagnosis of disturbed carbohydrate absorption

It is a drawback that the faecal lactic acid content measured is dependent on the laboratory method used With the method of Long both L and D lactic acid are determined whereas the enzymatic method using L lactate dehydrogenase measures L lactic acid only As Long's method is not entirely specific for total lactic acid it is incorrect to determine D lactic acid content indirectly by the difference between the two estimations However total lactic acid content i e the sum of L and D lactic acid may be determined specifically by gas chromatography so that the faecal D lactic acid may be accurately determined by the difference between the gas chromatographically determined L- and D lactic acid and the enzymatically measured L lactic acid

Application of these methods demonstrated the following

1 The faeces of breastfed infants sometimes contain only L lactic acid and sometimes besides L much D lactic acid

2 The faeces of most cases of disaccharidase deficiency contained only L lactic acid

3 A lactulose tolerance test performed on healthy infant resulted in L lactic acid only in the faeces

4 The faeces of a patient with protein losing enteropathy contained no lactic acid after alteration of the gut flora with Mexaform however lactic acid was present This was shown to be L lactic acid As lactic acid in this case could not have been derived from dietary carbohydrates it is suggested that it arose from breakdown products of mucus

J A Walker Smith (Sydney Australia) *Small bowel morphology in infancy and childhood A dissecting microscope study of 106 autopsies*

Little is known of normal small bowel morphology in children nor the extent of mucosal changes demonstrable on biopsy along the small intestine A *post mortem* study would extend such knowledge, but autolysis of the epithelium prevents adequate histological study If the autolysed surface cells of *post mortem* bowel are washed off the connective tissue cores of the villi are exposed and the overall arrangement of the villi and crypts can be studied using the dissecting microscope That the appearances seen are comparable to fresh biopsy specimens has been demonstrated by allowing autolysis of the epithelium of 14 biopsies to occur and then examining these in the same manner

Specimens from the duodenum proximal jejunum and distal ileum taken from 106 childhood autopsies performed at the Royal

Hooft *et al* of the methionine malabsorption syndrome (*Ann Paediat* 205 73 1965)

F Severi G Rondini S Zaverio & M Bruschelli (Pavia) *Prolonged neonatal hyperbilirubinemia and pregnane 3(Alpha) 20(Beta) Diol in maternal milk*

We carried out the determination of pregnane 3α 20β-diol (*a*) in milk of mothers whose infants showed prolonged neonatal unconjugated hyperbilirubinemia and (*b*) in milk of unselected mothers whose newborns were tested for bilirubinemia in the first weeks of life

(*a*) 7 breast fed infants with prolonged jaundice were observed for a period of 9 months Their ages ranged from 8 to 60 days and their total bilirubinemia from 3 8 to 22 6 mg/100 ml The direct reacting bilirubin was always very low (5–8 of the total) In all cases the determination of pregnane 3α 20β diol in maternal milk was performed The compound was present in 5 cases in 2 it was not In these 2 cases the newborns were 8 days and 26 days old the serum bilirubin was respectively 21 4 and 10 mg/100 ml In neither case other causes of prolonged hyperbilirubinemia were demonstrable The bilirubinemia fell quickly to normal values after discontinuing breast feeding

The administration of milk containing pregnane 3α 20β diol to a non jaundiced newborn aged 5 days for a period of 11 days did not cause a rise of serum bilirubin

(*b*) A systematic determination of pregnane 3α 20β-diol was performed in 17 samples of milk obtained from 17 mothers The compound was present in the milk of three mothers and their newborns showed prolonged unconjugated hyperbilirubinemia

On the basis of our investigation the presence of pregnane 3α 20β-diol in maternal milk seems quite frequent we found the compound in addition to 5 selected cases (with prolonged jaundice) in 3 out of 17 unselected samples of milk When the compound was present in maternal milk we always observed prolonged jaundice on the other hand in 2 cases of prolonged jaundice we were not able to demonstrate pregnane 3α 20β-diol in maternal milk

G Flatz (Chiang Mai Thailand) *Lactose intolerance in Thailand*

Intolerance to dietary lactose due to deficiency of intestinal lactase is wide spread in populations of African origin Recently an even higher incidence has been reported in small groups of oriental students in USA and Australia Our own study (lactose tolerance tests in over 200 individuals from northern and central Thailand) *shows that lactose intolerance has a very high* incidence in healthy adults In children the ability to digest lactose seems to disappear between the ages of 2 and 4 years With respect to the question of an adaptive or hereditary etiology of lactose intolerance we found that adults who took cow's milk for periods up to 3 years did not develop lactose tolerance Several children in northern Thailand who had taken milk regularly since weaning were also intolerant to lactose Tolerance tests in offspring of marriages between Southeast Asians and Europeans show a clear dimorphism and the results do not seem to correspond to the dietary history The results point at a genetic origin of lactose intolerance rather than an adaptive mechanism

C Polonovski J Badoual & I Navarro (Paris) *Congenital and familial digestive polyintolerance to proteins including human milk*

Two siblings with chronic multiple intolerance to dietary proteins including human milk proteins studied in collaboration with P Grenet are described

Frequent vomiting chronic diarrhoea and failure to thrive were noted from birth in Case 1 At the age of 25 days fat droplets were noted in the stools later steatorrhea and diarrhoea disappeared Although gluten had not

The positive test consisted of a remarkable increase of transformed lymphocytes after a 120 hour blood culture in the presence of 0 5 mg/ml milk proteins, in comparison with a control blood culture without antigen in some cases cultures with the milk fractions, lacto albumin and β_1 lacto globulin were carried out in addition *Immunologic lymphocyte response* was proved with a PHA stimulation in all cases We judged the positive result as the expression of an immunologic lymphocyte response to the antigen milk-proteins Beside the morphologic evaluation we considered the presence of a high number of mitoses obtained after a 3 hours incubation with Colcemid the presence of labelled cells and chromosomes with tritiated thymidine added to the culture in S phase the increase of beta glucuronidase in positive cultures lysosome enzyme which increases in the lymphocyte cultures added with PHA

We examined 39 children with this test In 11 controls of the same age as the patients there was no difference between the two cultures one with milk proteins and the other one without Also 4 children with coeliac disease were negative In 21 infants (4 to 12 months) suffering from chronic diarrhoea which seemed to be related with the milk feeding the results achieved were positive in 10 children and negative in 11 In 3 children suspected to suffer from milk allergy the response *has been positive in one of them the* test showed to be strongly positive to β_1 lacto globulin The substitution of powdered milk with sterilized and boiled milk caused a complete normalization of the infant

In the positive cases we found an increase of the lymphoblast index ranging from 4 to 20% in the cultures with milk proteins In 2 children examined during the acute phase of the disease the spontaneous *blast transforma*tion was higher than in all the other cases which *finding was probably* due to the antigenic stimulation *in vivo*

In comparison with several tests commonly used for the diagnosis of milk allergy the lymphoblastic transformation with milk proteins shows to be the more specific one Relying on our experience we propose this test for the diagnosis of cow's milk allergy

Ch S Bartsocas (Athens) J D Crawford & S O Thier (Boston Mass) *Demonstration of an independent transport system for L methionine in rat intestine and kidney*

The irregular elevation of urinary methionine excretion despite large losses of the other neutral amino acids in patients with Hartnup disease suggested to Efron (*New Eng J Med 272* 1058 1107, 1965) that methionine transport may be dependent upon an enzyme system different from that determining transport of the other neutral amino acids

In order to test this hypothesis L methionine transport was studied by measuring uptake of *the amino acid in rat kidney and intestine slices* in vitro Accumulation of L methionine against a chemical concentration gradient was shown The transport was found to be a saturable process in that the rates of accumulation failed to increase at high medium concentrations Its dependence in both tissues upon oxidative metabolism was evident from the inhibition of uptake under anaerobic conditions 2 4 dinitrophenol, however inhibited only the kidney accumulation Transport was *found to be relatively pH insensitive but critically dependent upon* the presence of sodium Normal rates of methionine accumulation were restored when tissue was replaced in sodium containing medium after incubation in sodium free sucrose but were subnormal after incubation in sodium free Tris medium *Of all amino acids tested in com*petitive inhibition experiments only ethionine and possibly L valine were found to inhibit its uptake

These results show that L methionine transport depends upon an enzyme system independent of the system(s) responsible for transport of the other neutral amino acids The findings are compatible both with Efron's hypothesis and with the subsequent description by

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normal child or according to the age it is only a little increased when compared with normal values in the literature (3) There is no significant correlation either with the intestinal albumin loss or with the *intensity of* the clinical picture This seems to indicate that the albumin synthesis is nearly at its maximum in normal subjects and that there is only a little compensation possible In case of enteric albumin loss the organism tries at first to keep a normal albuminaemia first by decreasing the metabolic catabolism of albumin and secondly by deplacing the albumin equilibrium from the extra to the intravascular pool hypoalbuminaemia appears only when a critical threshold is reached making any compensation mechanism no longer possible

REFERENCES

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The malabsorption was confirmed by the existence of steatorrhea The xylose test was twice positive the proline test once positive the lipiodol test once negative and once strongly positive

The histology of the small intestine showed atrophic changes of degree 1–2

As to the electronomicroscopic investigation the brush border of the enterocytes was nearly normal only seldom showing slight changes resembling those in celiac disease and in Down's syndrome Rather important changes were seen in the goblet cells which were only 1/4 their normal size very scarce about 1/5 their normal number and degenerated

Treatment with penicillamine seemed to be of some value Should the findings be confirmed in more such patients it is suggested to name this syndrome the oculo-cerebro-renal intestinal syndrome

O Saxl O Podhradska & J Tichy (Brno) *Gastrointestinal findings in Lowe's syndrome*

Ever since Lowe and co workers described the oculo-cerebrorenal syndrome much attention has been paid especially to the renal but also to the ocular and cerebral symptoms though no author has to the best of our knowledge ever mentioned gastro-intestinal disturbances besides anorexia vomiting and constipation Only lately Bartsocas and co-workers described a defect in intestinal amino acid transport in Lowe's syndrome similar to those in cystinuria Hartnup disease and familial iminoaciduria

In our patient we saw anemia and failure to thrive for long periods even at times when he had almost no protein loss in the urine In

P Pelkonen & J K Visakorpi (Helsinki) *Precipitins to cow's milk and gluten and the serum level of IgA in the follow up of coeliac patients*

The study was undertaken to determine whether during the later course of coeliac disease changes in the precipitating antibodies and in the serum level of IgA might provide information about the effects of the dietary treatment and its discontinuation Forty infants and children with coeliac disease were followed up for a minimum of 2 years A double diffusion technique and a single radial immuno-diffusion method were used

During treatment with a gluten free diet antibodies to cow's milk disappeared in only half of the patients whereas antibodies to gluten

been introduced in the child's diet, abdominal distension was present Pancreatic insufficiency α–β lipoproteinemia, disaccharidase deficiency chloride diarrhoea and exsudative enteropathia were excluded by appropriate tests During various dietary trials it was noted that meat was badly tolerated A soy formula, however was better tolerated but weight gain was inconsistent Human milk was not well tolerated and failure to gain weight persisted During 17 months diarrhoea alternated with constipation including an episode of intestinal obstruction caused by carrots The possibility of Hirschsprung's disease was definitely excluded by rectal biopsy There were slight biochemical signs of malabsorption Nevertheless there was no steatorrhea at this age Partial villous atrophy explained these symptoms The stools were always alkaline with a high fecal ammonia Amino acid chromatography of the stools revealed no selective amino acid malabsorption Moreover there was no post prandial rise of blood ammonia which excluded the protein intolerance described by Perheentupa & Visakorpi

A synthetic diet consisting of amino acids and a standard commercial protein hydrolysate was now given and this resulted in a rapid improvement of all symptoms and in weight gain Reintroduction of beef or horse meat cow's milk or ovalbumine was followed by immediate weight loss vomitings and in some instances diarrhoea Reintroduction of human milk caused the same symptoms The lactose of human milk had previously been removed by dialysis and the fat content had been reduced by centrifugation Using four different substrates no deficiency of dipeptidases was found in a jejunal biopsy specimen

Serum antibodies to lactoglobulin at the titer of 1/128 but no antibodies against maternal milk proteins were detected The lymphocyte transformation test was difficult to interprete

The brother of Case 1 was 12 years at time of investigation and he had shown similar symptoms with vomiting failure to thrive and diarrhoea already when he was breast fed Subsequently cow's milk and meat were also badly tolerated During his first year he presented eczema Within some years protein intolerance improved and meat could be given without harmful effects at 10 years

The early onset and the familial incidence suggest a genetic origin The parents are first cousins and there is eczema and asthma in the family

It seems that this disease is a separate allergic syndrome

The bad tolerance to human milk in which lactose and most of the lipids had been removed, was unequivocally demonstrated This distinguishes the congenital disorder of our patients from classical milk protein allergy which may be associated with intolerance to other nutritive proteins as previously described

H Gaze & A Donath (Berne) *Determination of intestinal loss and turnover of albumin in children with coeliac disease*

In a 14 month old child without gastro intestinal disease and in 4 children aged between 6 and 20 months presenting the clinical picture and the morphological alterations of intestinal mucosa typical for coeliac disease the turnover and enteric loss of albumin were studied All cases were normoproteinemic and had normal serum albumin The method was a modification of that described by Waldmann (1) and Kerr *et al* (2) using the simultaneous intravenous injection of two preparations of human serum albumin one labeled with ^{1} I and the other with ^{51}Cr The method and the results are compared with those of other authors

The results show that in coeliac disease the enteric loss of albumin is always 2 to 4 times higher than in the normal subject and correlates with the gravity of the clinical picture On the other hand the younger the child, the greater is the relative plasma volume The same applies to the albumin degradation rate however in coeliac disease it stays within the range of the

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(a) determination of faecal amine content in ulcerative colitis
(b) measurement of the low molecular weight fatty acids in the faeces in cases of protein losing enteropathy and small intestinal resection
(c) bacterial counts in combination with low molecular weight fatty acids estimation and faecal lactic acid content determination in a case of protein losing enteropathy

2 Bacterial metabolites absorbed in the colon and excreted in the urine The bacterial metabolites of tryptophane which appeared in the urine in a case of tryptophane malabsorption were described

3 Once the presence of protein malabsorption has been established one must investigate possible aetiological factors In this connection the determination of peptidase activity in gut mucosa biopsy specimens in a case of coeliac disease and a case of generalized malabsorption syndrome due to mucoviscidosis was reported

B Lindquist (Lund) *Sugar induced diarrhoea* Two main types of sugar induced diarrhoea exist (1) deficiency of disaccharidase activities (2) deficiency of the transport systems for monosaccharides This is however more or less an artificial division as there is a close relationship between digestion and absorption of sugars these processes should rather be regarded as two steps of one common function

During recent decades there has been some discussion whether sugar induced diarrhoea is mainly of osmotic or fermentative nature Although both mechanisms may exist it could be concluded from the considerable amounts of sugar excreted with faeces in patients with glucose galactose malabsorption that the diarrhoea is mainly of osmotic origin This has been confirmed by Launiala in intubation studies in patients with lactose malabsorption the unabsorbed disaccharide (lactose) caused a considerable movement of water into the intestinal lumen

Next to the pathophysiology of the disturbed absorption Glucose tolerance tests as well as intubation studies have shown that a small but definite absorption of glucose ocurs in glucose-galactose malabsorption in upper jejunum it amounts to about 10 Under normal conditions galactose has been found to be absorbed slightly faster than glucose when these sugars are given separately In glucose-galactose malabsorption the opposite is true on oral sugar loading tests the fecal excretion of galactose was found to be roughly 2½ times that of glucose indicating that in this condition the cells of the intestinal mucosa are almost impermeable to galactose

A most interesting question is whether we are dealing with a defect bound to the intestinal mucosa or a more generalized disturbance Studies in our department have shown that in cases of glucose-galactose malabsorption there are signs of a disturbed intermediate carbohydrate metabolism This seems however to be related to a secondary carbohydrate deprivation rather than to the basic disease itself it is also reversable The same phenomenon was observed in a malnourished patient with cystic fibrosis of the pancreas

Although lactase deficiency may be an exception the acquired forms of sugar malabsorption are probably secondary to unspecific lesions of the intestinal musoca The nature of such a lesion and in what way such a lesion affects the mucosa is however still unclear During recent years this problem has become actualized as there have been described several cases of so-called intractable diarrhoea in early infancy in which all kinds of monosaccharides are slowly absorbed In one case observed by us an intestinal biopsy performed in the acute phase showed in certain areas partly destructed villi and villi of somewhat irregular shape and furthermore an increased number of inflammatory cells especially plasma cells were observed The diarrhoea was interpreted as caused by an unspecific lesion of the intestinal mucosa primarily inflammatory changes due to an enteral infection but with subsequent damage of

nearly always disappeared during the first few months The initially elevated serum level of IgA normalized rapidly Thirteen patients were followed up beyond reinstitution of a gluten-containing diet In ten of these recurrence of the mucosal lesion was verified within 2 to 10 months and this was accompanied by symptoms in 6 cases Significant elevation of the IgA level ensued in 6 of the 10 patients and precipitins to cow s milk and gluten reappeared in 4 and 8 patients respectively Only one of the relapsers showed none of these signs In 3 patients the small intestinal mucosa remained normal Nevertheless one of these reacted to the change of diet with and elevation of the IgA level and in another precipitins to gluten were again detectable

Determination of the serum IgA level and precipitating antibodies to cow s milk and gluten is of value in the follow up of coeliac patients Disappearance of antibodies may be a favourable sign indicative of normalization of the mucosal lesion Persistence or reappearance of antibodies to gluten during a gluten free diet is indicative of non adherence to the diet After discontinuation of the diet reappearance of antibodies and/or elevation of the IgA level is strongly suggestive of a relapse of the mucosal lesion which is not, however always detected by these tests

EUROPEAN SOCIETY FOR PAEDIATRIC RESEARCH

EUROPEAN SOCIETY FOR PAEDIATRIC GASTROENTEROLOGY

Joint Meeting in Interlaken September 16 1969

J H van der Kamer (Zeist) & H A Weijers (Utrecht) *The diagnosis of protein malabsorption*

Dietary protein digestion is facilitated by heating during the process of preparation prior to consumption Heat denatures the proteins thus facilitating subsequent enzymatic breakdown

Proteolysis of dietary protein in the stomach results from the action of pepsine HCl the bonds split being mostly those of phenylalanine leucine and tyrosine Of the pancreatic enzymes trypsine splits the bonds with lysine and arginine chymotrypsine those with tyrosine phenylalanine and tryptophane and the carboxypeptidases A and B split the bonds with carboxyl terminal amino acids Proteolysis is completed in the intestinal mucosal cell by carboxypeptidase A amino peptidase prolinase prolidase dipeptidase and probably other as yet unrecognized enzymes

Disturbances which may occur are disturbed activation of the above named enzymes disturbed gastro intestinal motility disturbed innervation and/or perfusion of the gastro intestinal tract and disturbed intra cellular function Small intestinal resection the blind loop syndrome and protein losing enteropathy in addition will lead to abnormal protein digestion This will all lead to protein malabsorption resulting in an excess of peptides reaching the ileocoecal region leading to an increase of the bacterial flora and disturbing the normal bacterial pattern The result is production of excessively large quantities of normal and also abnormal bacterial metabolites and toxins as well as osmotic changes within the gut lumen

Diagnostic investigation will therefore have to be focused on

1 The gut flora and its metabolites in the faeces The following examples were discussed

kinase a proteolytic enzyme secreted by the mucosa of the small intestine Enterokinase converts the proenzyme of trypsin (trypsinogen) into active trypsin trypsin then activates the other pancreatic zymogens (chymotrypsinogens and procarboxypeptidases)

Thus enterokinase is the key enzyme in this process and enterokinase deficiency must result in a complete failure to activate pancreatic zymogens

So far 2 cases of intestinal enterokinase deficiency are known (1 2) In both patients the disease manifested as a severe disturbance of protein digestion with diarrhoea from birth failure to thrive anaemia and severe hypoproteinaemia

The biochemical evidence that the primary defect of protein digestion in these patients is due to enterokinase deficiency is provided by three observations

1 In the duodenal fluid the activities of the proteolytic enzymes trypsin chymotrypsin and carboxypeptidase are undetectable or extremely low The addition of enterokinase to the fluid in vitro results in a rapid appearance of normal activities of all three enzymes From this is must be concluded that the pancreatic zymogens are secreted normally into the duodenum but cannot be activated

2 After Gel filtration of duodenal fluid enterokinase activity can be directly measured It is undetectable in the patients duodenal fluid

3 No enterokinase activity is detected in the patients duodenal mucosa obtained by peroral biopsy

Enterokinase deficiency is probably a congenital deficiency of one sigle intestinal enzyme Evidence that it is a primary defect rather than the effect of a more generalized small bowel disease is provided by the following observation At the age of 17 months one of the patients was reinvestigated At this time she had following a substitution therapy with pancreatic extracts containing enterokinase fully recovered from the secondary effects of protein malabsorption A duodenal biopsy was taken which was morphologically normal and had normal lactase activity Enterokinase activity however was still undetectable in the mucosal homogenate

REFERENCES

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Two patients with intestinal enterokinase deficiency have been studied both were girls who presented with diarrhoea from birth and failure to thrive Only one of the children developed hypoproteinaemia Investigations showed absence of proteolytic activity in the duodenal juice chymotryptic and carboxypeptidase activity appeared after the addition of trypsin The addition of a small amount of enterokinase to the juice resulted in rapid and complete activation of pancreatic proteolytic enzymes normal levels of trypsin chymotrypsin and carboxypeptidase appeared in the duodenal juice showing that the precursor forms of all these enzymes were present In the first patient enterokinase activity was assayed after gel filtration of the juice on a Sephadex G 100 column and was not detected enterokinase activity was similarly absent in the soluble fraction of a homogenised duodenal biopsy specimen although it was present in control mucosal biopsies from patients with coeliac disease Duodenal biopsies from both children were normal to light and electron microscopy and had normal disaccharidase activities

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the absorption mechanisms causing sugar as well as protein intolerance

G Meeuwisse (Lund) *Renal glucosuria in glucose–galactose malabsorption*

The glucosuria of 3 fasting patients with glucose–galactose malabsorption was measured quantitatively with a sensitive method for the detection of glucose in urine The concentration of glucose was in the range in which Clinistix gives varying results (15–40 mg/100 ml) or sometimes slightly higher This corresponded to a moderately increased excretion of glucose per unit time which was not found in four of the parents of the patients Two of the patients were subjected to forced diuresis by drinking large quantities of water The glucose concentration fell to values as low as 3 mg/100 ml which was however higher than in controls with diuresis of the same magnitude The maximal reabsorption capacity of glucose (Tm_G) was found to be normal in 3 patients and before the attainment of Tm_G there was not much increase of the glucosuria

The patients are probably capable of transporting glucose actively in the renal tubuli by a mechanism other than the intestinal mucosa It was speculated that in normal individuals the mechanism responsible for the bulk transport of glucose in the small intestine has some task but only a minor one in the kidney

T Lindberg (Malmo) & B Karlsson (Lund) *Developmental changes in intestinal dipeptidases of foetal and neonatal pig as related to changes in the ultrastructure of the mucosal cells*

The dipeptidase activities (units/mg N) on L alanyl L glutamic acid L alanyl L proline and glycyl L leucine are very low in porcine intestinal mucosa from early foetal development (2 cm C–R length) The activities increase uniformly up to the stage of 14 cm C–R length The L alanyl L proline dipeptidase activity thereafter remains at about the same level throughout the whole developmental period The other two activities however increase considerably from the stage of 14 cm C–R length The values registered in newborn piglets not fed with colostrum are 2–3 times higher than those from slaughtered pigs However these activities decrease abruptly and significantly after the newborn pig has ingested colostrum

The electron microscopic studies reveal a striking parallelism between enzyme levels registered and appearance of microvilli in the absorptive cells Microvilli are practically lacking in fetuses of 2 cm C–R length but begin to differentiate in the 4–5 cm fetuses With proceeding foetal development they reach step by step the appearance earlier noted in the mucosa of newborn piglets (Mattison & Karlsson, 1966)

The results obtained complete and illustrate earlier findings in man and rat (Lindberg 1966) Correlation between structure of mucosal cells and magnitude of dipeptidase activity thus exists in rat (Lindberg & Owman, 1966) and pig Moreover in man it has been shown that intestinal dipeptidase activities are significantly reduced in coeliac disease (Lindberg *et al* 1968) where the structure of the mucosal cells i e the microvilli are known to be affected

The decrease of the dipeptidase activities after colostrum ingestion cannot yet be satisfactorily explained Experiments performed *in vitro* did not demonstrate any direct inhibitory effect of porcine colostrum on the activities while other experiments *in vivo* have shown that the decrease is connected in one way or another with the ingestion of colostrum

B Hadorn (Berne) *Intestinal enterokinase deficiency A newly recognized disorder of protein digestion in early infancy*

Proteolytic enzymes of the pancreas are stored in the gland as inactive precursors (zymogens) They become activated only after the pancreatic secretion has reached the duodenum The key enzyme in this activation process is entero-

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The clinical and laboratory features of trypsinogen deficiency disease appear identical with those of our patients and this diagnosis was origially made in our first case We suggest that enterokinase should be assayed in patients with trypsinogen deficiency disease

A simple screening test for enterokinase deficiency has been developed The sample of duodenal juice to be tested which is deficient in tryptic activity is incubated with juice from a patient with known exocrine pancreatic insufficiency the latter contains enterokinase but no trypsin After incubation the mixture is assayed for trypsin in enterokinase deficiency trypsin will appear

C Polonovski & H Bier (Paris) *Pseudo trypsinogen deficiency due to a lack of intestinal enterokinase*

Together with Dr Allison we studied an infant presenting during the first month of life with *diarrhoea anemia and severe hypoproteinemia* The duodenal juice showed an isolated lack of *trypsin activity Similar cases have been de*scribed by Townes (1 2) and Morris *et al* (3) and have been interpreted as trypsinogen deficiency disease In our case some findings made us suspect that not a complete lack but rather an abnormality of trypsinogen was present The gelatine film test for trypsin activity in stool was strongly positive The duodenal juice appeared to have activity towards the synthetic trypsin substrate B A N A (benzoylarginine naphthylamide) but did not react with other substrates like haemoglobin gelatin and B A E E (benzoylarginine ethylester) We now attempted to activate the patient s pancreatic zymogens with bovine crystallised trypsin using short activation times *and without adding cal*cium With this procedure chymotrypsin and *procarboxypeptidases were readily* activated but no additional trypsin was found suggesting that *the patient s trypsinogen* was not activated

At this point of the investigation we learned of a similar case (4) in which intestinal enterokinase deficiency was found to be the cause of this syndrome We therefore attempted to activate the *patient s duodenal juice* with a duodenal juice containing enterokinase but no trypsin (we obtained this from a patient with complete pancreatic insufficiency) With this method the patient s trypsinogen was readily activated Incubation of the patient s duodenal juice with normal duodenal mucosa also resulted in trypsinogen activation, an ileal mucosal homogenate *however failed to activate* Direct measurements of enterokinase activity in duodenal juice and mucosa of the patient were performed by Hadorn (5) and confirmed enterokinase deficiency

Although trypsinogen of animal origin can be activated by crystalline trypsin in vitro, human trypsinogen appears to resist the activation with exogenous crystalline trypsin On the *other hand, the activation of human trypsinogen* by enterokinase is rapid Furthermore this *process is facilitated after addition of* calcium and dialysis of the duodenal juice

Since it is difficult to activate human trypsinogen with exogenous bovine trypsin (a method used by Townes & Morris in an attempt to prove trypsinogen deficiency) we would like to raise the question whether the cases described by these authors may have suffered from the duodenal enterokinase deficiency rather than from a defect of pancreatic *trypsinogen* Figarella has recently described two human trypsinogens Therefore if trypsinogen deficiency exists one would have to admit that a common precursor of these two zymogens is involved

An additional defect which we *found in* our patient was deficiency of phospholipase Phos*pholipase depends for its* activation from trypsin It is not unlikely that non activation of phospholipases has caused the transitory steatorrhea which is observed in enterokinase deficient infants

REFERENCES

1 Townes P L *J Pediat 66* 275 1965
2 Townes P L Bryson M F & Muller G *J Pediat 71* 220 1967

3 Morris M D & Fisher D A Amer J Dis Child 114 203 1967
4 Hadorn B Tarlow M J Lloyd J K & Wolff O H Lancet I 812 1969
5 Hadorn B Personal communication

Ch M Anderson (Birmingham) *The value of intestinal biopsy in the investigation of small intestinal disorders*

Since the introduction in the late 1950s of peroral intestinal mucosal biopsy procedures the understanding of a number of small intestinal disorders has greatly increased This technique has enabled the examination of "living intestinal mucosa by various means—structural histological biochemical and by tissue culture Its first contribution was the demonstration of the altered villous pattern in coeliac disease ("flattened mucosa) This appearance was thought at first to be specific to that disease but further evaluations showed it to be present from other reasons than wheat gluten intolerance A wide appraisal of the findings of many workers can now clarify the actual diagnostic value of alterations in villous structure revealed in biopsies The occasions in which this investigation is of real clinical value were discussed

The technique has proved of great value in the study of enzymatic activities in the mucosa particularly in relation to the digestion of sugars For some years the technique was of research value in this field but again an appraisal of findings now clarifies the limits of its usefulness in clinical practice

Specific structural alterations of the mucosa in certain rare disorders of small intestinal function such as intestinal lymphangiectasis and abetalipoproteinaemia can also be revealed

The technique still has research potential in the delineation of other disorders of enzyme function particularly in regard to protein digestion and absorption and also to the immunological aspects of the small intestinal mucosa In these areas clinical applications is still not fully clarified

J Rey (Paris) *Disturbances of intestinal fat transport*

Disturbances of intestinal fat transport include all the anomalies concerned with the entry of monoglycerides and free fatty acids into the cells of the intestine resynthesis of triglycerides the formation of chylomicrons and the discharge of fats into the intercellular spaces and their passage in the lymphatics

Firstly a review was given of the principal steps of digestion and absorption of fats emphasizing the role of bile salts in their solubilization as micelles So far as resynthesis of triglycerides is concerned the regulatory mechanisms inside the cells of the intestine the mechanisms of formation of chylomicrons and the factors which influence their distribution between the lymphatic and portal venous system were discussed

Secondly a review was given of all the pathological conditions in which a disturbance of intestinal fat transport occurs They comprise deficiencies of bile salts and malabsorption syndromes with atrophy of the intestinal mucosa Furthermore are included defects in chylomicron formation either secondary to a β lipoproteinaemia or idiopathic both remarkable examples of intercellular block of fat transport facilitating the understanding of certain details of normal transport mechanisms Finally anomalies of the lymphatics with stasis or lipid in the lamina propria and at the base of the absorptive cells themselves belong to this group of disorders

R D G Milner (Kingston Jamaica) *Hormonal and metabolic interrelationships in malnutrition*

Malnourished Jamaican infants aged between 8 and 22 months and weighing 3 2 to 8 6 kg were studied Clinically most babies had marasmus a few had marasmic kwashiorkor The greatly increased growth rate of such infants made them suitable subjects for a study of the hormonal changes associated with growth

Plasma concentration of glucose, free fatty acids (FFA) α-amino nitrogen, insulin and growth hormone were measured *in response* to either intravenous glucagon (0 1 mg/kg) or a mixture of the 10 essential amino acids given orally (0 5 g/kg) Venous blood samples were taken before and at 3 10 30 and 60 min after glucagon and before and at 15, 30 60 120 and 180 min after oral amino acids

Each test was *performed shortly after admission* (sick infants) and again 6–10 weeks later when the children were clinically recovered (well infants) Fasting levels of plasma FFA and growth hormone were higher in sick infants while insulin levels were lower

The plasma glucose response to glucagon *was greater in well infants In both groups there* were similar small rise in insulin at 3 min FFA and α amino nitrogen levels fell Plasma growth hormones levels rose to a maximum at 10 min and subsequently fell

The plasma amino nitrogen level rose to a peak at 30–60 min following oral amino acids *No consistent change in plasma glucose or* insulin levels occurred FFA levels fell to a nadir at 60 min and then rose above fasting levels In both sick and well infants there was a fall in plasma growth hormone levels over the *first 30–60 min to a level which was main*tained for the next 2 hours

Conclusion In these malnourished infants high plasma growth hormone levels could be further raised by intravenous glucagon or depressed by oral amino acids Plasma insulin levels were low and responded poorly to glucagon both on admission and after recovery

R Gitzelmann T Bachi H Binz, J Lindenmann & G Semenza (Zurich) *Localization of rabbit intestinal sucrase isomaltase with ferritin antibody conjugates*

Rabbit intestinal sucrase isomaltase complex was isolated and precipitating antisera were obtained from guinea pigs Antibody ferritin conjugates were prepared After the removal of the enteric surface coat by prefixation and *tryptic digestion*, pieces of mucosa were exposed to the conjugates Ferritin of antibody ferritin conjugates was localized with the electron microscope It was hown that the antibody recognized sucrase isomaltase at the surface of the undisrupted microvilli It was concluded that the sucrase isomaltase complex in the rabbit *was membrane bound i e that is consti*tuted an integral part of the outermost layer of the microvillus membrane

A Holzel (Manchester) *A rare type of* E coli *gastroenteritis*

From the end of December 1968 until May 1969 an unusual form of infantile gastroenteritis was encountered 80 cases during that period showed all degrees of severity but serious forms predominated and carried a high fatality rate, particularly among infants with gross congenital malformations In spite of the maintenance of electrolyte and acid base balance absorptive capacity of the alimentary tract was completely disrupted and any attempt of oral feeding lead to a relapse of vomiting and diarrhoea Intravenous *alimentation had to be continued* for periods up to six weeks In patients who re*covered intolerances for mono* and disaccharides persisted for many weeks In the babies who succumbed liver failure had become irreversible

The aetiological agent is assumed to be *E coli* 0114 which has previously only *been found* in two outbreaks The organism was resistant *to ten* of the common antibiotics but sensitive to Gentamicin and Colomycin Since the determination of the antibiotic sensitivity the administration of the two antibiotics to all patients admitted with *E coli* 0114 infection has greatly *affected the course of the disease* There were no further deaths and intravenous alimentation in the graver forms was required only for much shorter periods Sugar malabsorption was limited to lactose

D H Shmerling (Zurich) & M Shiner (London) *The response of the intestinal mucosa to the intraduodenal instillation of gluten in patients with coeliac disease during remission*

The response of the intestinal mucosa to the intraduodenal instillation of boiled gluten was evaluated in two children 5 1/12 and 8 1/12 year old with coeliac disease in remission following a gluten free diet for 3½ years Biochemical studies serum IgA assays as well as dissecting light and electron microscope investigations of small intestinal mucosal biopsy specimens were performed before the instillation of gluten and 2 48 and 96 hours after

No clinical biochemical or immunological changes occurred during the period of investigation When examined with the dissecting and the light microscope the mucosa showed after the instillation only slight changes consisting mainly of oedema and increased cellular infiltration Ultrastructural changes were evident in the mucosa only 48 and 96 hours after the instillation and consisted of thickening widening and coarsening of the epithelial basement membrane Similar changes of the basement membrane of small blood vessels in the lamina propria associated with endothelial proliferation also became evident Only subsequently an increased degeneration and shedding of villous epithelial cells through their detachment from their basement membrane as well as burrowing of round cells mainly lymphocytes through the villous epithelial cells appeared The lamina propria was then heavily infiltrated with lymphocytes plasma cells and some mast cells

These changes suggest that gluten does not exert a direct toxic effect on the villous epithelium They are consistent with pathological changes taking place primarily in the lamina propria possibly as a result of a delayed type immunological response

Further similar studies on other patients are in progress

H Loeb P Petit, M Vainsel M L Buyle & A Piepsz (Brussels) *Congenital chloride diarrhoea*

A 6-year-old boy presented with congenital chloride diarrhoea metabolic alcalosis (pH 7 51 total CO 25 mEq/l) hypochloremia (83 mEq/l) and hypokaliemia (3 0 mEq/l) and furthermore weight and growth retardation

Renal function was characterized by a reduced glomerular filtration rate (inuline clearance 59 ml/min/1 73 m) isosthenuria with unresponsiveness to A D H aciduria and a lowered phosphorus threshold

Histological study of the kidney showed severe glomerular sclerosis

The physiopathological mechanisms of this disease were discussed

EUROPEAN SOCIETY FOR PAEDIATRIC GASTROENTEROLOGY

Meeting in Interlaken September 18 1969

Round table discussion *Diagnostic criteria in coeliac disease*

Panel H A Weijers B Lindquist Ch M Anderson J Rey D H Shmerling J K Visakorpi B Hadorn R Gruttner

The basis for the discussion was the result of the questionnaire sent out by Dr Visakorpi (see below) to the members of the Society and to some other pediatricians Among them there had been general agreement upon one of the main questions i e that coeliac disease is due to gluten intolerance and that is usually (but not necessarily) present with symptoms and signs of malabsorption The intestinal mucosa shows constantly typical structural alterations

Plasma concentration of glucose free fatty acids (FFA) α amino nitrogen insulin and growth hormone were measured in response to either intravenous glucagon (0 1 mg/kg) or a mixture of the 10 essential amino acids given orally (0 5 g/kg) Venous blood samples were taken before and at 3 10 30 and 60 min after glucagon and before and at 15 30 60 120 and 180 min after oral amino acids

Each test was performed shortly after admission (sick infants) and again 6–10 weeks later when the children were clinically recovered (well infants) Fasting levels of plasma FFA and growth hormone were higher in sick infants while insulin levels were lower

The plasma glucose response to glucagon was greater in well infants In both groups there were similar small rise in insulin at 3 min FFA and α amino nitrogen levels fell Plasma growth hormones levels rose to a maximum at 10 min and subsequently fell

The plasma amino nitrogen level rose to a peak at 30–60 min following oral amino acids No consistent change in plasma glucose or insulin levels occurred FFA levels fell to a nadir at 60 min and then rose above fasting levels In both sick and well infants there was a fall in plasma growth hormone levels over the first 30–60 min to a level which was maintained for the next 2 hours

Conclusion In these malnourished infants high plasma growth hormone levels could be further raised by intravenous glucagon or depressed by oral amino acids Plasma insulin levels were low and responded poorly to glucagon both on admission and after recovery

R Gitzelmann T Bachi H Binz, J Lindenmann & G Semenza (Zurich) *Localization of rabbit intestinal sucrase isomaltase with ferritin antibody conjugates*

Rabbit intestinal sucrase isomaltase complex was isolated and precipitating antisera were obtained from guinea pigs Antibody ferritin conjugates were prepared After the removal of the enteric surface coat by prefixation and tryptic digestion pieces of mucosa were exposed to the conjugates Ferritin of antibody ferritin conjugates was localized with the electron microscope It was hown that the antibody recognized sucrase isomaltase at the surface of the undisrupted microvilli It was concluded that the sucrase isomaltase complex in the rabbit was membrane bound i e that is constituted an integral part of the outermost layer of the microvillus membrane

A Holzel (Manchester) *A rare type of* E coli *gastroenteritis*

From the end of December 1968 until May 1969 an unusual form of infantile gastroenteritis was encountered 80 cases during that period showed all degrees of severity but serious forms predominated and carried a high fatality rate particularly among infants with gross congenital malformations In spite of the maintenance of electrolyte and acid base balance absorptive capacity of the alimentary tract was completely disrupted and any attempt of oral feeding lead to a relapse of vomiting and diarrhoea Intravenous alimentation had to be continued for periods up to six weeks In patients who recovered intolerances for mono- and disaccharides persisted for many weeks In the babies who succumbed liver failure had become irreversible

The aetiological agent is assumed to be *E coli* 0114 which has previously only been found in two outbreaks The organism was resistant to ten of the common antibiotics but sensitive to Gentamicin and Colomycin Since the determination of the antibiotic sensitivity the administration of the two antibiotics to all patients admitted with *E coli* 0114 infection has greatly affected the course of the disease There were no further deaths and intravenous alimentation in the graver forms was required only for much shorter periods Sugar malabsorption was limited to lactose

gliadin or special gliadin hydrolysates are neither practical nor entirely reliable for this purpose because of variations in preparation procedures

4 *Definition of gluten free diet* A gluten free diet must not contain wheat rye barley or oats (French blé seigle orge avoine German Weizen Roggen Gerste Hafer) Of these only wheat and rye are known with certainty to be harmful to patients with coeliac disease Barley is supposed to be harmful Oats may well be harmless but this point needs further investigation Buckwheat and millet as well as corn (maize) and rice are harmless The maximum tolerable amount of original wheat protein in wheat starch preparations made for patients with coeliac disease can not be defined as no studies on this aspect have been done

Coeliac like manifestations may be caused in young babies also by milk protein intolerance and disaccharidase deficiency In these cases diagnostic confusion is possible when the diet contains wheat besides milk protein and disaccharides Furthermore it is quite possible that susceptibility to gluten is induced more easily when wheat is administered to very young babies Hence the meeting agreed that the modern trend of introducing wheat into the diet before the age of 4 months is undesirable

G W Meeuwisse

APPENDIX

AN INTERNATIONAL INQUIRY CONCERNING THE DIAGNOSTIC CRITERIA OF COELIAC DISEASE

A summary of results Edited by J K Visakorpi Helsinki

At the request of the council of European Society for Paediatric Gastroenterology (ESPGA) a questionnaire was prepared to acquire information on how the diagnosis of coeliac disease is made in various hospitals today The opportunity was taken of asking simultaneously about the treatment of this disease The questionnaire was sent to 50 colleagues including all the members of ESPGA working in different hospitals and in addition to some other pediatricians interested in gastroenterology In all 33 questionnaires were returned The respondents were as follows Ch Anderson (Birmingham) S Auricchio (Napoli) H Berger (Innsbruck) J A Black (Sheffield) D Boda and L Szabo (Szeged) I J Carr (Belfast) M Davidson (New York) K Dietel (Jena) E Eggermont (Louvain) R Grüttner (Hamburg) J R Hamilton (Toronto) D C Heiner (Torrance) T Lindberg (Malmo) B Lindquist and G Meeuwisse (Lund) J K Lloyd (London) H Loeb (Bruxelles) I Mestyan (Pécs) V Mengoli (Verona) S Nordio (Trieste) D Nusslé (Lausanne) C Polonovski (Paris) J Rey and J Jos (Paris) E Rossi B Hadorn and J Gaze (Bern) O Saxl (Brno) D H Shmerling (Zurich) A Storen (Oslo) R R W Townley (Melbourne) J A Walker Smith (Sydney) H A Weijers J H van de Kamer and E A K Wauters (Utrecht) L Wranne (Örebro) R Zetterstrom (Stockholm) G Zoppi (Ferrara) and J K Visakorpi (Helsinki)

The first part of the questionnaire related to the definition of coeliac disease A simple model was given for definition coeliac disease is a malabsorption syndrome due to gluten intolerance This model was generally accepted Some wished to add the characteristic biopsy findings to the definition However a great deal of confusion arose as a result of the question concerned with the permanency of gluten intolerance Fifteen respondents said they had evidence of the existence of a transient gluten intolerance 13 had doubtful evidence and five had no such evidence at all A slight majority (18) considered that the transient form if it existed should be excluded from coeliac disease There was rather close unanimity that patients suffering from one systemic disease such as diabetes mellitus or intolerance to other foodstuffs such as cow's milk should be called coeliacs if they otherwise fulfill the criteria

The main question the diagnostic criteria of coeliac disease has been divided into two parts the basic clinical and laboratory criteria and the testing of gluten intolerance

The answers concerning diagnostic symptoms signs and laboratory results varied a great deal Table 1

A majority considered, therefore an intestinal biopsy to be indispensable for the diagnosis

At the round table the discusion was centered around four questions Agreement upon them was desired *in order to be able to com* pare future research findings from different European centres

1 *Definition of coeliac disease* It was decided that the diagnosis of coeliac disease should be restricted to patients with *permanent* gluten intolerance Although most of the panel and the audience believed that transient gluten intolerance (if it exists) is relatively rare it was decided that more facts about such conditions were required For the moment a patient with absent or almost absent villi (so called subtotal villous atrophy) who shows definite improvement on a gluten free diet cannot be designated as having coeliac disease before he has been proved *to normalize entirely* (or almost so) on dietary treatment not only clinically but also histologically and subsequently to relapse after the reintroduction of gluten

2 *The basic clinical and laboratory criteria for the diagnosis of untreated coeliac disease* Most infants and small children with coeliac disease show failure to thrive abdominal distension and frequent bulky stools but it is clear that asymptomatic cases exist some of whom *might have their first symptoms in adult life* No laboratory test is entirely reliable but the faecal excretion of fat is seldom within the normal range Many centers seemed to apply rather liberal standards for this test Van de Kamer stressed that with a 5 day-stool collection and the method described by him indicated as method B and implying fat extraction and subsequent saponification the upper normal limit for children on a normal diet is 3 g per day In clinical practice absorption tests tests of nutritional status and exclusion tests for other diseases e g cystic fibrosis bacterial or parasitic infections etc have to be *performed* The only decisive criteria however are the abnormal morphology of the small intestinal mucosa its normalization on gluten withdrawal and the reaction on reintroduction of gluten

The biopsy should be taken in the distal duodenum or proximal jejunum Examination of the specimen under a dissecting microscope will usually show the characteristic flat mucosa *in untreated coeliac disease but for* complete evaluation histological examinaton is necessary Up to now no specific enzyme deficiency (di peptidase disaccharidases, etc) has been found in coeliac disease

3 *Testing gluten intolerance* Several of the panelists with extensive experience of patients with coeliac disease were inclined to believe that formal testing of gluten intolerance may be unnecessary but in practice after years of treatment many patients must be allowed to try gluten at least for a while If on such a trial recurrence of the typical mucosal lesion can be demonstrated it is usually easier to convince the patients or their parents of the necessity to continue the diet throughout life

Initially the clinically judged improvement on a gluten free diet should be supported with simple absorption tests Most clinicians do not reintroduce gluten again during the initial treat *ment of the patient (provocation test)* There was agreement upon the point that in order to decide whether a patient has permanent gluten intolerance the intestinal mucosa must first become normal before the effect of gluten is *studied again In children on a* strict gluten free diet normalization of the mucosa may take one or two years Before reintroduction of gluten a new biopsy should be taken On resumption of a normal diet recurrence of disease is usually slow or may even be silent Before a patient is classified as having been suffering from *transient* gluten intolerance the intestinal biopsy should *still be* normal *two years after* the reintroduction of gluten At present none of the members of the Society has such final proof of the existence of cases with transient coeliac disease either because suspected cases *have not been biopsied* at the beginning of the disease or because they have not yet been followed up for a sufficient length of time

The testing of gluten intolerance *should* be done with wheat or (wheat) gluten At present

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

SCANDINAVIAN ASSOCIATION OF PAEDIATRIC SURGEONS

Fifth Meeting Sept 17–19 1969 Vedbaek Denmark

WINKEL SMITH MEMORIAL LECTURE

Preben Plum (Copenhagen Denmark) *On medical and surgical treatment of cerebral palsy*

SYMPOSIUM ON INTERSEX

Henning Andersen (Copenhagen Denmark) *Pediatric and endocrinological aspects*

P Fogh Andersen (Copenhagen Denmark) *Surgical aspects A survey of 20 years experience*

Surgical treatment of intersex conditions varies from rather simple operations to more complicated reconstructions and is a fascinating subject on the borderline of pediatric surgery general surgery gynecology urology and—not least—plastic surgery This fact appears clearly from a series of intersex patients operated upon during the last 20 years partly in Rigshospitalet and Queen Louise s Children s Hospital partly in Diakonissestiftelsens Hospital (Deaconess Hospital) Copenhagen

Following the classification of Henning Andersen the largest number of patients in our series belonged to the groups of male pseudohermaphroditism (complete or incomplete testicular feminisation and female pseudohermaphroditism (adrenogenital syndrome)

In testicular feminisation (Morris syndrome) when discovered in infancy or childhood the gonads could either be transposed into the abdomen for protection awaiting the future development of the patient at puberty or they could be removed and later replacement hormone therapy instituted If in addition there is a vaginal aplasia we recommend a construction of an artificial vagina after the age of puberty our method of choice has been inlay skin grafting using a modified McIndoe technique Congenital absence or hypoplasia of the breasts in partial testicular feminisation could be corrected successfully by implantation of prostheses (Cronin or Arion) For psychological reasons we don t tell the male pseudohermaphrodites about the true character of their gonads or genetic sex Correctly treated most of these patients will be able to have a normal life as women and get happily married

In the management of female pseudohermaphroditism (adrenogenital syndrome) a close cooperation between the pediatrician and the surgeon is of greatest importance It has been our experience that in cases of enlarged penis like clitoris this should not be preserved an infant of doubtful sex belonging to this group will never develop a well functioning penis The child is better reared as a girl and a plastic operation on the clitoris performed at an early stage before the age of 3 or 4 years We prefer a subtotal amputation of the clitoris i e removal of the corpora cavernosa leaving the glans or part of it attached to its blood supply An accompanying hypoplasia of the vagina is preferably corrected later

The true hermaphodites with both ovarian and testicular tissue are very rare No case happened to be included in our operative series during 20 years

So-called psychic hermaphroditism trans

Table 1 *Diagnostic symptoms signs and investigations*

Number of respondents who consider particular symptom signs or lab tests as essential for diagnosis	Symptoms signs and laboratory tests
More than 10	Onset of symptoms after introduction of cereals prolonged diarrhoea pale bulky and offensive stools failure to gain weight and height abdominal distention abnormal faecal fat abnormal xylose absorption normal sweat tests abnormal mucosa
More than 15	Prolonged diarrhoea failure to gain weight abnormal faecal fat abnormal xylose absorption normal sweat test abnormal mucosa
More than 20	Failure to gain weight abnormal faecal fat normal sweat test abnormal mucosa
More than 25	Failure to gain weight abnormal faecal fat abnormal mucosa normal sweat test
More than 26	Abnormal faecal fat abnormal mucosa
More than 27	Abnormal faecal fat

shows how many respondents considered the particular symptoms sign or laboratory tests diagnostic According to this the most important symptom is the failure to gain weight followed by prolonged diarrhoea and failure to grow Abdominal distension was the most important sign The most important laboratory and other examinations are faecal fat the intestinal biopsy sweat test and the D xylose test These results showed a pattern of symptoms signs and laboratory findings typical of coeliac disease but it was difficult to extract from the questionnaires what everyone considered to be the minimal requirements for diagnosis and this was the original intention of the question With two exceptions everybody listed some symptoms and everybody again with the exception of the same two listed some laboratory tests All but five regarded biopsy as essential

The separate testing of gluten intolerance was considered necessary The commonest method applied by everybody was that of observation during the administration of gluten free diet Of 33 28 confirmed the effect of gluten exclusion by laboratory tests and 17 by biopsy Eighteen also suggested that a provocation test should be made after initial improvement

Table 2 *The number of intestinal biopsies and perforations reported*

Type of instrument	No of biopsies (approx)	No of perforation
Crosby & Watson	3 895	6
Rubin tube	592	1
Hydraulic instrument	350	1
Sebus tube	100	—
	4 937	8 (0 16)

although the questionnaire failed to elicit correct answers on exactly when such provocation should be introduced

The suggested lengts of treatment on gluten free diet were as follows life long by 7 until growth ceases by 6 many years by 5 / to 2 years by 11 Thirty two respondents excluded wheat from the diet 30 rye 25 oats 26 barley 9 buckwheat and 9 millet

The questionnaire also provided some details of the technique of certain laboratory investigations The determination of faecal fat was the most widely applied absorption test and was usually performed by the classical method of van de Kamer The collection period varied from 2 to 6 days and carmine was often used as the faecal marker in balance studies The other tests which indicate fat absorption were not used very much the commonest of them was the microscopic examination of stool fat Radioactive methods widely used for adults were not applied in pediatric hospitals For examination of the absorption of carbohydrates D xylose absorption tests where the ones most commonly used The glucose tolerance test was also used to a rather large extent In 18 instances the D xylose excretion test was applied The dosage had large variations but similar normal values were adapted Nine applied the D xylose tolerance test

The questionnaires also provided some data on biopsies performed (Table 2) Eight perforations were mentioned representing 1 6 per 1 000 The Crosby capsule the instrument employed most widely was also the commonest cause of perforations Very few other complications were reported bleeding prolonged pyloric spasm transitory apnoea on introduction of the tube and impaling of the outer spring of the Crosby capsule in the oesophagus

It is important to realise that the results of this inquiry do not solve the problem of diagnostic criteria of coeliac disease and do not impart to us the final truth about the disease However interesting information is provided on how the different pediatricians make the diagnosis today and how they treat the disease

and tubes The determination of sex chromatin and hormone analysis give further support to the diagnosis

Surgical correction of the external genitalia should be started at 3 years of age when the child is not yet sexually determined and it should be completed at 4–5 years of age For surgical treatment of the internal genitalia I consider the age of 10–11 years most suitable

In correction of the external genitalia of a male individual straightening of the penis is performed where curvature of erection exists The broad fibrous plate on the underside of the penis is broken by means of small incisions when satisfactory straightening has been achieved the defect is reconstructed with the aid of two flaps of penile skin At the second session the terminal urethra construction is made with a continuous buried strip of epithelium from the meatus out to the glans as presented by Denis Browne

When the vagina uterus tubes and streak gonads occur in a male individual an attempt is made to extirpate these As a rule removal of the tubes and gonads does not cause any difficulties However extirpation of the vagina and uterus involves the risk by reason of their anatomical position that adjacent organs will be injured consequently this form of intervention should not be made Curiously enough a remaining vagina causes no trouble or infections The series of patients from the Department of Plastic Surgery Sahlgrenska Sjukhuset included 7 male individuals with a vagina 2 (aged 39–45 years) 2 (aged 13–18 years) and 3 (aged 1–5 years) In all of these persons repeated quantitative cultures have shown <1 000 bacteria per ml of urine

In general abdominal testes are defective in spermatogenesis but as a rule they produce adequate androgenic hormone for normal or nearly normal development of secondary masculine sex characteristics Moreover there is the risk of malignant tumours developing if the testes are allowed to remain If a testis with a deferent duct is present an attempt should be made to transpose it through the inguinal canal outside the body cavity When testis and tubes are present transposition is impossible

G Fock & O Qvist (Stockholm Sweden) *Surgical correction of the hypertrophic clitoris*

A method for conservation of the clitoris in cases of adrenal virilization in girls and in other groups of female pseudohermaphrodites is discussed as an alternative to amputation of the phallus In the operation described the enlarged clitoris is lifted up by a pubic incision and the cavernous bodies after undermining of the skin and the prepuce are subcutaneously fixed to the suprapubic muscular fascia The glans of the clitoris will be partially covered and the skin and the redundant prepuce form the labia minores The patients in the authors series of patients were operated upon at varying ages preferably at the time for reconstruction of the perineum and the vaginal opening in cases with labial fusion and urogenital sinus The results seems to be anatomically acceptable in most of the cases treated in the way mentioned above

FREE PAPERS

Ove K Mjølnerød & Trond Kluge (Oslo Norway) *Familial occurrence of the megaduodenum syndrome with primary degeneration of duodenal muscle layers*

A report is given on the occurrence of megaduodenum in three brothers aged 17 years 9 years and 19 *months In* the oldest patient, 2 laparotomies with freeing of adhesions failed to improve the condition Following the establishment of a duodeno-jejunostomy satisfactory drainage of the stomach and duodenum was obtained Histological examination of biopsies from the duodenal wall revealed an extensive degeneration and fragmentation of all muscle layers The small vessels and the ganglions of the submucous and myenteric plexus showed a normal appearance External compression of the duodenum could be ruled out as a causative mechanism and generalized

vestism and trans sexualism might in selected cases need surgical management, but never before adult age

In a few patients with Turner's syndrome we have been able to correct the pterygium colli by means of a z-plastic procedure, and some Klinefelter patients have had an operative correction of the gynecomastia

In addition to the typical intersex patients a series of hypospadies of all degrees have been treated—altogether nearly 500 the method of choice has been Denis Browne's urethroplasty During the last 2 years a number of chromosome analyses were made in our hypospadias patients so far without any abnormalities however not even in some severe perineal hypospadias

Gunnar Grotte Gabriel Reuterskiold & Örjan Grimås (Uppsala Sweden) *Intersex at the Pediatric Surgical Service in Uppsala*

R Bjordal & O Knutrud (Oslo Norway) *Intersex*

During a 20 years period (1950–1969) a total of 43 patients with intersex have been admitted to The Children's Clinic Rikshospitalet Oslo The series comprises

A Adrenogenital syndrome	24
(*a*) Male sex role	8
(*b*) Female sex role	16
B Male pseudohermaphrodites	14
(*a*) Male sex role	7
(*b*) Female sex role	7
C Female pseudohermaphroditus	1
D True hermaphrodite	1
E Agonadism	3
Total	43

Ad A (*a*) In this group 6 patients including 2 siblings had pure male sex organs Two patients had bi sexual external genital organs and ovaries They had been brought up as males and their female pseudohermaphroditism was diagnosed too late (8 ¹/ and 16 years respectively) for a change in their social sex role

Ad B The decision of the social sex role has principally been based upon the predominant external genital appearance—including urethro-(vagino)graphy and sino urethroscopy In 5 patients the social sex role has been changed from male to female after years of observation They are

	Changed
1 male pseudohermaphrodite	3 years old
1 male pseudohermaphrodite	5 ¹/ years old
1 adrenogenital syndrome	6 ¹/ years old
1 true hermaphrodite	2 ¹/ years old
1 agonadism	2 ¹/ years old

In each particular case this serious decision has been based upon intimate collaboration between pediatricians the psychologist paediatric psychiatrist ocial worker and paediatric surgeon

During the last 10 years the surgery needed has been performed by the paediatric surgeon and we feel this point to be of great value In this connection, it may be stressed that during recent years we have practised transposition of the clitoris instead of clitoridectomy

L Avellan (Gothenburg Sweden) *Surgical treatment of gonadal dysgenesis*

Disturbances in the development of the genital organs and in gonadal differentiation may result from deviation in the chromosome formation endocrine disturbances or from a combined form of these viz genetic defects in the enzyme system with ensuing failure of the cells in the rudimentary genital organs to the hormone normally produced

A correct diagnosis should be established during the neonatal period, when the sex of an individual is also determined Chromosome analysis is very valuable Nevertheless I consider that exploratory laparotomy with biopsy from the gonads is traumatizing and should be avoided and replaced by well-conducted urethrocystography which provides reliable information on the presence of the

diaper) are now being tried out in the ward We have applied the technique in about 30 cases with different types of stomas The result can be considered good and encouraging In most patient skin irritation soreness leakage and odour disappeared

We find the method a considerable practical advance which is in many cases preferable to the usual collecting bag system Contact has been made with a manufacturer who plans to produce the unit commercially

O Knutrud & H C Sommerschild (Oslo Norway) *Extrophia vesicae urinariae*

Since 1960 26 patients with true exstrophy of the bladder have been treated at the Paediatric Surgery Service Rikshospitalet Oslo The results of primary bladder reconstructions are extremely disappointing as are the results from other centres according to published reports

True continence is achieved only in some individual cases In view of this and upon the anatomical situation of defective bladder and the defective nerve supply defective pelvis ring defective pelvic floor and defective abdominal wall it is proposed that primary reconstruction should be carried out only in the very few cases of female patients with a large bladder

In most cases operation should be postponed until the patients is 2 years of age a cutaneous ureter ileo stomy should then be performed or maybe rather an implantation of the ureters to the rectosigmoid If this rectal conduit fails this may later be converted into a cutaneous conduit

This policy will enable reduction of the number of operations which the child has to undergo the genital area is left for better plastic repair and the risk of irreversible renal damage is minimized

P Fogh Andersen (Copenhagen Denmark) *Congenital duplication and diverticulum of the male urethra*

Two boys with the rare congenital malformation duplicatio urethrae were operated on One had an accessory meatus on the dorsum of the glans surrounded by a hemispherical prominence simulating an extra micro-glans an urethral channel could be traced about 2 cm backwards from the accessory opening Primary excision was very easily made and a good cosmetic result achieved

The other case was a complete duplication from the corona of the glans through the dorsal part of the entire penis up to the bladder neck, and persistent "incontinence and fever attacks were present After excision of the distal major part of the accessory urethra no incontinence persisted the fever attacks almost disappeared and the cosmetic result was excellent Transvesical excision or ligation of the proximal part might have been better however leaving the distal accessory urethra open

A case of large diverticulum formation of the major part of the penile urethra associated with chronic urethritis in a boy aged 2 years was treated in two stages first splitting up the ventral wall of the urethra and excision of the superfluous mucosa thus producing an artificial hypospadias at a second stage of Denis Browne urethroplasty was performed with satisfactory cosmetic and functional result

Jaakko Elo (Helsinki Finland) *Refluxing ureters in children*

Since September 1965 150 children with pyelonephritis have been examined at the out patient urological clinic of Aurora Hospital of these patients 124 were girls and 26 boys

Vesicoureteral reflux was found in 53 girls and 12 boys This gives an incidence of 43 for reflux in this series The reflux material also includes four girls without any urinary infection who attented for the treatment of enuresis The total series consists of 69 children with vesicoureteral reflux Of these 28 children were treated operatively and 41 conservatively

Twenty-six patients were operated upon by application of the Politano-Leadbetter tunnel

neuromuscular disease could also be excluded

It is suggested that this type of megaduodenum is due to a primary muscular degeneration and dysfunction which has previously not been reported It seems to follow a hereditary pattern and may be connected with similar dysfunctions of the bladder and large intestine

Three patients all with intrinsic obstruction

B Brook Jacobson & C M Madsen Odense Denmark) *Neonatal duodenal obstruction*

During the years 1957–1968 20 cases of neonatal duodenal obstruction were treated at Odense County and City Hospital surgical department K and pediatric department J

Ten patients had extrinsic obstruction (6 boys and 4 girls) and ten intrinsic obstruction (6 boys and 4 girls) Five patients with intrinsic obstruction had severe associated anomalies Two had Downs syndrome

In all cases the extrinsic obstruction was caused by malrotation with duodenal compression in 8 cases associated with volvulus of the small intestine They were all treated with Ladd s operation In no case was intestinal resection necessary

Of the cases with intrinsic obstruction annular pancreas was found at operation in 7 cases 2 cases had duodenal stenosis and 1 case diaphragmatic obstruction In 5 cases duodeno jejunostomy was performed and in 4 cases duodeno duodenostomy On the case with diaphragmatic obstruction a plastic operation was performed

Three patients, all with intrinsic obstruction died postoperatively One with a birthweight of 1,500 g died three days after the operation The two patients with Down s syndrome died 5 1/ and 8 months after the operation

Two patients treated by duodeno duodenostomy were successfully operated upon for intestinal obstruction caused by adhesions 5 and 7 months after the primary operation

Fourteen patients have been followed up from 3 to 11 years after the operation None of these has had gastrointestinal disturbances

A G Coran, R Bjordal, S Eek & O Knutrud (Oslo Norway) *The Surgical management of small intestinal aganglionosis*

Six cases of aganglionosis of the entire colon and the terminal ileum were reported

To be published in Surgery Oct. 1969

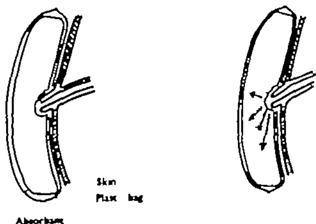
N Öhre Nilsen (Copenhagen Denmark) *Abdominal wall defects experimentally produced in chicken embryos*

R Bjordal (Oslo Norway) *A new method for the collection of urine in urostomies*

In children with some kind of urinary diversion the method most commonly applied is that with the collecting bag

We all know the great practical problems mainly arising from leakage and skin irritation with this equipment At the Rikshospital Paediatric Surgical Service Oslo a new principle, invented by the mother of a myelomeningocele patient has been employed during the last 6 months This new technique, entitled Urosorb has the following characteristics A water absorbing paper diaper is placed in a thin plastic bag with a suitable opening for the stoma Ureterostoma (ileostoma) is put into the opening and the plastic bag is then placed on the abdomen with slight pressure The urine will now drip into the absorbing material and be abducted—the principle being the Wick effect (see figure)

Various fixation belt arrangements for application of the absorbing unit (plastic bag+



B L Lindstrom O Lindfors M Westerlund & B Kuhlbeck (Helsinki Finland) *Kidney transplantation in children—a follow up*

In September 1968 at the annual meeting of The Scandinavian Association of Paediatric Surgeons presentation was made of a series of 4 children who had received kidney transplants (*Acta Paediat Scand 58* 422 1969) One of the children died in postoperative bleedings from gastrointestinal ulcers with the transplant still completely normal What happened to the three other children during the succeeding years?

Case 1 Girls age 11 at time of transplantation follow up 31 months Increase in height 3 cm and weight 24 kg Mammae and pubic hair in accordance with age No menarche

Case 2 Girl age 11 at time of transplantation follow up 16 months Increase in height 2 cm and weight 14 kg Mammae and pubic hair in accordance with age No menarche

Case 3 Boy age 13 at time of transplantation follow up 16 months Increase in height 7 cm and weight 4 kg No Cushing symptoms

All 3 patients are in good condition blood pressure is normal and retinal changes have regressed totally No osteoporosis has been observed All the children attend school move actively and are no longer bound to dialysis centres The routine examinations are made at local hospitals

Ervin Struve-Christensen & Jørgen Lejerstofte Jensen (Copenhagen Denmark) *Winkelmanns operation in boys*

Since 1898 the name of Winckelmann has been connected with partial excision and aversion of the hydrocele sac

In Gentofte Hospital—Copenhagen—76 boys under 15 years of age were treated for congenital hydrocele of the testis or funicel in the period 1960 to 1967

Sixty six of the hydroceles were located in the testis 42 on the right side 18 on the left side and 6 were bilateral

Ten funicular hydroceles were evenly distributed with 5 on either side

A follow up of 72 patients has been effected after a postoperative period of from 1 to 7 years

Twenty seven of the patients were operated on by application of Winckelmann s principle and 11 of these developed recurrence of the hydrocele mostly within the first years They were all subjected to re-operation with ligation of the patent processus vaginalis without further recurrence

Primary ligation of the patent processus vaginalis was performed in another 27 patients one of these had a recurrence

Thirteen patients were treated with both ligation of the patent processus vaginalis and Winckelmann s operation No recurrence was observable

A funicular hydrocele was excised in 6 patients and other methods were applied in another 3 cases

Consequently Winkelmann s operation can not be recommended in child surgery

We now perform ligation of the patent processus vaginalis in the way described by Mc Kay Fowler & Barnett in 1959

C M Madsen & H W Vennits (Odense Denmark) *Traumatic haemobilia*

A report is made on a case of traumatic haemobilia in a boy aged 5 years After moderate trauma in the region of the liver the primary symptoms disappeared rapidly A week later pain resembling hepatic colic occurred accompanied by melaena The diagnosis was established from this typical clinical picture and confirmed by arteriography The cavity in the liver was opened at operation, and the damaged vessels and biliary passages were sutured The subsequent cource was uncomplicated

A review of the literature has led to the conclusion that arteriography is an important pre-operative investigation and that operation should consist either of opening the cavity with suture of vessels and biliary passages or par

lin technique The operation was performed on 40 ureters the reflux had disappeared in 31 ureters The results in respect of 8 operated ureters have not yet been checked Heminephroureterectomy was performed in 2 cases and cutaneous ureterostomy in only 1 case

The original method of Politano-Leadbetter has proved to be safe and reliable of my patients subjected to operation by this technique two developed a postoperative stricture These two were reoperated upon and the stricture has disappeared In one of these the reflux recurred unilaterally after the reoperation

Of the 20 patients successfully operated upon chemotherapy was discontinued in 15 these children are progressing well without any drugs In 5 cases, a recurrence of infection necessitated the reinstitution of chemotherapy

The reflux disappeared in 12 patients treated conservatively of whom 7 are getting well without chemotherapy Chemotherapy is still being administered to the remaining 5 to ensure a good result or to treat recurrences of infection

Niels Krøigaard (Copenhagen Denmark) *Repeated urinary tract infection and dysfunction of the lower urinary tract in infancy and childhood*

A new physiological method is presented including micturition cinematography combined with simultaneous, suprapubic intravesical pressure—flow recording This method makes it practicable to evaluate the lower urinary tract in infancy and childhood with great accuracy The values of intravesical pressure and urinflow are given, along with a description of the syndrome, urethral dysfunction in female children Bladder neck obstruction has not been demonstrated in female children moreover it has not been possible to prove genuine lower urinary tract obstruction in them Lower urinary tract obstruction in male children is demonstrated as valvulae urethrae congenitas and strictura urethrae Bladder neck obstruction in male children is an extremely rare disease, and is consequently without numecal importance for the relatively large group of patients who present trabeculation reflux and repeated urinary tract infection Longstanding infection did not give rise to bladder neck obstruction Bladder outlet obstruction in childhood is the cause of only a small part of the repeated urinary tract infections

Jan Gierup (Stockholm, Sweden) *The urinary flow in normal infants and children*

The urinary flow was studied in 216 boys and 180 girls, aged one day to 16 years, by the use of the method described previously in which the urinary flow is converted into air flow which is recorded via pneumotachograph—differential pressure manometer

Considerable variations were observed in the micturition pattern of different individuals With increased micturition volume the flow curve displayed a plateau formation in the age group 4–13 years this plateau seems to be typical of children It occurred with decreasing incidence in older children

The maximum flow was positively correlated to the volume of voided urine as in adults In girls no differences were demonstrable between age groups In boys of 0–14 years, the increase in maximum flow with volume was more pronounced than in boys aged 4–16 years In the age group 4–16 years, the increase in maximum flow with volume in girls showed a more pronounced increase than in boys

An attempt was made to correlate the maximum flow with height, weight body surface and age respectively The correlation coefficients were small and about the same for all the parameters Thus the maximum flow was correlated mainly with the micturition volume

Growth seems to exercise little influence upon normal emptying of the bladder Micturition dynamics in normal infants and children seem to be determined principally by physiological and hydrodynamic factors

B L Lindstrom O Lindfors M Westerlund & B Kuhlbeck (Helsinki Finland) Kidney *transplantation in children—a follow up*

In September 1968 at the annual meeting of The Scandinavian Association of Paediatric Surgeons presentation was made of a series of 4 children who had received kidney transplants (*Acta Paediat Scand 58 422 1969*) One of the children died in postoperative bleedings from gastrointestinal ulcers with the transplant still completely normal What happened to the three other children during the succeeding years?

Case 1 Girls age 11 at time of transplantation follow up 31 months Increase in height 3 cm and weight 24 kg Mammae and pubic hair in accordance with age *No menarche*

Case 2 Girl age 11 at time of transplantation follow up 16 months Increase in height 2 cm and weight 14 *kg* Mammae and pubic hair in accordance with age No menarche

Case 3 Boy age 13 at time of transplantation follow up 16 months Increase in height 7 cm and weight 4 kg No Cushing symptoms

All 3 patients are in good condition blood pressure is normal and retinal changes have regressed totally No osteoporosis has been observed All the children attend school *more* actively and are no longer bound to dialysis centres The routine examinations are made at local hospitals

Erwin Struve Christensen & Jørgen Lejerstofte Jensen (Copenhagen Denmark) *Winkelmann's operation in boys*

Since 1898 the name of Winckelmann has been connected with partial excision and eversion of the hydrocele sac

In Gentofte Hospital—Copenhagen—76 boys under 15 years of age were treated for congenital hydrocele of the testis or funicel in the period 1960 to 1967

Sixty six of the hydroceles were located in the testis 42 on the right side 18 on the left side and 6 were bilateral

Ten funicular hydroceles were evenly distributed with 5 on either side

A follow up of 72 patients has been effected after a postoperative period of from 1 to 7 years

Twenty seven of the patients were operated on by application of Winckelmann's principle and 11 of these developed recurrence of the hydrocele, mostly within the first years They were all subjected to re-operation with ligation of the patent processus vaginalis without further recurrence

Primary ligation of the patent processus vaginalis was performed in another 27 patients one of these had a recurrence

Thirteen patients were treated with both ligation of the patent processus vaginalis and Winckelmann's operation No recurrence was observable

A funicular hydrocele was excised in 6 patients and other methods were applied in another 3 cases

Consequently Winkelmann's operation can not be recommended in child surgery

We now perform ligation of the patent processus vaginalis in the way described by Mc Kay Fowler & Barrett in 1959

C M Madsen & H W Venmits (Odense Denmark) *Traumatic haemobilia*

A report is made on a case of traumatic haemobilia in a boy aged 5 years After moderate trauma in the region of the liver the primary symptoms disappeared rapidly A week later pain resembling hepatic colic occurred accompanied by melaena The diagnosis was established from this typical clinical picture and confirmed by arteriography The cavity in the liver was opened at operation and the damaged vessels and biliary passages were sutured The subsequent cource was uncomplicated

A review of the literature has led to the conclusion that arteriography is an important pre-operative investigation and that operation should consist either of opening the cavity with suture of vessels and biliary passages or par

tial resection of the liver with removal of the lesion If no other treatment is possible, ligation of the hepatic artery can be performed

H C Sommerschild, T Kluge & A Flatmark (Oslo Norway) *Sinusoidal obstruction as a cause of portal hypertension in children and young adults*

Two patients with portal hypertension were operated upon at 7 and 21 years of age respectively The portal vein was patent in both cases and the liver appeared normal on gross examination

Light and electron microscopic studies of wedge liver biopsies revealed a marked hypertrophy of Kupffer and narrowing of the sinusoidal lumina There was a heavy perisinusoidal fibrosis with obliteration of the space of Disse The hepatic parenchymal cells and the portal triads were normal

Pre and post sinusoidal obstructions could be excluded in both patients The observations suggest that in the present the portal hypertension is induced by a flow obstruction at the sinusoidal level These findings seem to add a new aspect to the pathogenesis of the "idiopathic portal hypertension (To be published in Surgery)

Hans Chr Børresen Arnold G Coran & O Knutrud (Oslo Norway) *Parenteral feeding of newborns undergoing major surgery*

The ultimate success of operations on newborns often depends upon complete parenteral feeding for extended periods of time A normal newborn requires about 100 cal/kg body weight in 24 hours which must be supplied in a fluid volume of about 140 ml In human milk about 50% of the calories are provided by fat Since this has not been possible in parenteral feeding hitherto strongly hypertonic solutions of carbohydrate have been used to cover the *infant s high* caloric need This hypertonicity necessitates the use of the superior or inferior vena cava for infusions Although peripheral phlebitis is thus avoided the tendency to glucosuria and osmotic diuresis persists

The present communication describes an improved parenteral feeding programme in terms of amounts given/kg body weight in 24 hours The corresponding figures for breeast feeding are stated below in parenthesis The metabolic performance of the feeding programme has been analysed by multiple balance studies

Fat intake is increased to 4 g (6 g) administered as 20 Intralipid® Vitrum The proper clearance of this fat from the serum is ensured by the addition of 300 I U of heparin/kg body weight to the amino acid solution This small amount of heparin was shown to normalize the otherwise increased triglyceride level

A quantity of 3 g of amino acids (1 8 g) is given as a commercial solution of crystalline *l* amino acids, viz Aminofusin L® forte Pfrimmer The Aminofusin is mixed with 10% invertose the total amount of carbohydrate beeing *12 g (10 5 g)/kg Both a multi* vitamin preparation and electrolytes are added to this mixture The total content of K /kg body weight is 1 75–2 75 mEq (1 8 mEq) of Ca^{++} 1 mEq (1 mEq absorbed from mother s milk) and of phosphate 1 15–2 20 mmoles (0 6 mmoles absorbed from mother s milk) The Aminofusin provides 0 15 mEq Mg^{+} (0 17 mEq absorbed from mother s milk)

Balance studies in four patients for periods of 3 days to 4 weeks show that the feeding programme sustains positive nitrogen balances even in the immediate postoperative period of the same order of magnitude as in normal infants in the second week of life, i e about 200 mg N retained/kg 24 hours The retention of K^{+} was close *to 4 mEq/ K /g N* This is compatible with the synthesis of normal soft tissues the K /N element ratio of which is between 3 and 4 mEq/g The supply of Mg^{+} was however probably somewhat too low since the Mg^{+}/N retention ratios were considerably lower than the Mg^{++}/N element ratio of soft tissues which is usually close to

0 6 mEq/g N Breast feeding sustains a Mg^{+} /N retention ratio close to 0 6 mEq/g which would probably not be sufficient if the deposition of Mg in the skeleton were not limited by a short supply of phosphorus in mothers milk The P/N retention ratio varied between 54 and 207 mg P/g N The P/N retention ratio during breast feeding is close to 80 mg P/g N while bottle feeding increases the ratio to between 170 and 250 The P/N element ratios of soft tissues are usually between 60 and 85 mg P/g N About 90 of the calcium was retained as would be expected in view of the relatively liberal supply of phosphorus The retention of calcium confirms our view that infants as opposed to adults confined to bed require Ca Mg and phosphorus to cover the needs of their growing skeleton

A practical advantage of the feeding programmes outlined above is that small veins tolerate the infusions for between 2 and 4 days This results from the reduction in the hypertonicity achieved through the liberal use of fat and from the simultaneous infusion of the fat emulsion and the mixture of amino acids etc Adjustment of the pH of the latter solution might further improve the tolerance

Jørgen Bech Hansen & Svend Arne Pedersen (Odense Denmark) *Intussusception in infancy and childhood*

During the period 1936–65 196 cases of intussusception in children were treated The duration of the symptoms in 63 3 % of the cases was less than 24 hours and primary barium enema was used in 173 cases 5 patients were operated upon primarily under the diagnosis intussusception and 18 patients were operated upon for other conditions A second operation was necessary in 68 of the 173 cases In 10 cases the intussusception was induced by organic lesions in the bowel all of these being of a benign nature A follow up examination showed that the rate of recurrence calculated as a decrement series was be 7 8% There is no statistically significant difference in the recurrence percentage following barium enema and that following primary and secondary operation The fatality of the total material was 3 6% After 1949 95 cases were treated without fatalities

Primary operative treatment was applied in only a few cases in which the patient was dehydrated and the abdomen distended or with peritoneal reaction In the remaining cases the intussusception was primarily treated by barium enema

Th Ehrenpreis J Gierup & R Lagercrantz (Stockholm Sweden) *Treatment of regional enterocolitis in children and adolescents*

A series of 35 patients with regional enterocolitis is presented Of these 31 were operated upon 27 had colonic localization of the disease 24 of them with the small intestine engaged as well Medical therapy had no more than a temporary effect and most of the patients 23 were subjected to surgical therapy by reason of chronic progressive disease with grave efforts upon weight growth and the development of puberty

The recurrence rate was high—about 50 Wide spread disease seemed to have an unfavourable prognosis Resections in the distal part of the colon with anastomosis is not recommended in cases with perianal and perirectal manifestations The importance of careful preoperative assessment of the extent of the lesions and of a radical excision is stressed Notwithstanding the high recurrence rate surgery is our best choice of treatment in the majority of patients at present

Johannes E Bock (Odense Denmark) *Anorectal atresia with rectocloacal fistula*

Rectal atresia with the persistence of cloaca account for about 3 of anorectal malformations The anomaly is a "high atresia and is attributable to disturbance in the mechanism of the division of the embryonic cloaca

A female child with high rectal atresia, double vagina with two separate uteri and a persistent cloaca with openings from the bladder the two vaginas and rectal fistula, is presented There were no associated anomalies except for a persistent omphalo mesenteric duct

The therapeutic procedure is demonstrated especially the pull through operation with sacro abdomino perineal approach

The prognosis for fecal and urinary continence is discussed

G R Wallgren

Aurora Hospital, Helsinki, Finland

NEW BOOKS RECEIVED

G Blix (ed) *Nutrition in preschool and school age* Symposia of the Swedish Nutrition Foundation VII 154 pp Almqvist & Wiksell Stockholm 1970 Sw kr 45 –

E J Brewer Jr (ed.) *Juvenil rheumatoid arthritis* Major Problems in Clinical Pediatrics vol VI 231 pp illus W B Saunders Philadelphia London and Toronto 1970 £5 6s

G Henneberg (ed) *Masernschutzimpfung* Abhandlung aus dem Bundesgesundheitsamt Heft 8 91 pp Springer Verlag Berlin Heidelberg and New York 1969 DM 22 US $6 10

V A Cowie *A study of the early development of mongols* 110 pp Pergamon Press Oxford 1970 US $8 00

R Gourdeau (ed) *The hemophiliac and his world* Bibliotheca Haematologica no 34 214 pp illus Proc 5th Congr World Fed of Hemophilia Montreal 1968 Karger Basel and New York 1969 DM 56 US $13 45

O Oetliker (ed) *Nephrologie im Kindesalter I* Pädiatrische Fortbildungskurse für die Praxis vol 27 117 pp illus Karger Basel and New York 1970 DM 30 US $7 20

A Prill *Die neurologische Symptomatologie der akuten und chronischen Niereninsuffizienz* 177 pp illus Springer Verlag Berlin Heidelberg and New York 1969 DM 64 –

P F Bray *Neurology in pediatrics* 514 pp illus Year Book Medical Publ Inc., Chicago Ill. 1969 US $23 50

Ch N Barnard & V Schrire *Die Chirurgie der häufigen angeborenen Herzmissbildungen* 152 pp illus Springer Verlag Berlin Heidelberg and New York 1969 DM 12 80 US $3 20

F Unterharnscheidt D Jachnik & H Gott. *Der Balkenmangel* Monographien aus dem Gesamtgebiete der Neurologie und Psychiatrie Heft 128 232 pp illus Springer Verlag Berlin Heidelberg and New York 1969 DM 68 –

R Delarveleye *Le problème de l'hérédité dans les syndromes épileptiques* 236 pp Expansion Scientifique Française Paris 1969 43 F

E Goth *Pathophysiologie und Klinik der Fettsucht* 140 pp Akadémiai Kiadó Budapest 1969 US $6 00

C C Mabry I E Roeckel R E Gevedon & J A Koepke *Recent advances in pediatric clinical pathology* 247 pp illus Grune & Stratton New York and London 1969 Price not given

XXII congrès de l'Association des Pédiatres de langue française Strasbourg 1969 Vol 1 *Alimentation du prématuré* Vol 2 *Réanimation du nouveau-né* Vol 3 *Mucopolysaccharidoses et sphingolipidoses* Expansion Scientifique Française Paris 1969 Price not given

E Davis *Rheumatic fever Clinical ecological and familial aspects* 160 pp Thomas *Springfield Ill* 1969 US $9 75

M Stefanini (ed) *Progress in clinical pathology* vol II 385 pp illus Grune & Stratton New York and London 1969 US $19 50

I Schulman (ed) *Advances in pediatrics* vol 16 469 pp Year Book Medical Publ Inc Chicago Ill 1969 US $15 00

BOOK REVIEWS

N S Scrimshaw C E Taylor & J E Gordon *Interactions of nutrition and infection* World Health Organization Geneva 1968 329 pp £2 14s US $9 00 sFr 27 00

Only few physicians will be surprised to learn that kwashiorkor is rarely due only to malnutrition infection usually of the intestinal tract plays an important additional role On the other hand the fatality rate of infectious disease is greatly influenced by reduced host resistence due to nutritional deficiencies The book barely elucidates the mechanisms behind these well known facts as far as hitherto investigated It is a comprehensive review and a synthesis of the available literature on the clinical epidemiological and experimental aspects concerning the two kinds of interaction involved Effect of infection on nutritional state and effect of nutrition on infection The interaction may be synergistic antagonistic or absent

The negative effect of infection on nutrition is mainly encountered as the result of acute diarrhoea or of chronic infestation by parasites especially those invading the gut For separately grouped nutritional deficiencies the effect of infection by different micro organisms and helminths have been systematically listed Malnutrition is usually synergistic with infectious disease When virus protozoa or helminths are involved however antagonism sometimes does occur which seems to be very rarely the case in bacterial or rickettsial infections Fungal infections have hardly been mentioned because of the scarce information available A particular chapter deals with the weanling diarrhoea a good example of the complex nature of the interactions described in other parts of the book

The book is of major importance for physicians and other scientists engaged in the efforts of improving public health in the large regions of the world where malnutrition is frequently recognized Even for clinicians working in more prosperous areas valuable information can be extracted For ten years the authors have jointly been collecting and evaluating the literature in this field Their final choice of nearly 1500 references tells something about the amount of reading behind this No 57 of the World Health Organization monograph series

Gunnar Meeuwisse

F Vasella (ed) *Aspekte der pädiatrischen Neurologie* Pädiatrische Fortbildungskurse für die Praxis No 24 113 pp S Karger Basel New York 1968 sFr /DM 26 —

This volume is one in a series written for postgraduate courses in pediatrics It contains good rather short reviews of some of the actual problems in pediatric neurology F Vasella Bern has written a very instructive and informative chapter on neurologic examination of infants with tables of motor and mental development The surveys of cerebral tumours in childhood by E Zander Lausanne and of hydrocephalus by M Bettex Bern are easy to read relatively short but still with all the important aspects on the problems Other subjects which are reviewed are retinal changes in degenerative disorders of the nervous system acute hemiplegias in childhood leucodystrophies and head injuries Most of the chapters are followed by a short list of up to date references but some have very few or none at all which is a pity for the interested postgraduate reader

Ingrid Bjerre

D Jelliffe *Infant Nutrition in the Subtropics and Tropics* Second ed 335 pp illus World Health Organization Geneva 1968 £2 14s

This monograph from WHO was first published 1955 and during the following 13 years there has been a slowly increasing awareness among people in the developed countries of the world of the need for them to participate in the struggle against malnutrition and diseases in the underdeveloped countries For those who are interested in understanding more about these problems this monograph gives a good introduction After a short historical survey about infant feeding since ancient civilization there is a presentation of infant feeding practices in about 50 less developed countries all over the third world Some common patterns of infant feeding are seen in most of the countries e g an increased frequency of the failure of lactation among mothers in the lower socio economic group living in urban areas often associated with unsuccessful attempts at bottle feeding Animal milk seems to play very little part in the feeding of the majority of poorer infants Eggs and other protein food are also inadequately used Carbohydrate in some form is often the sole dominating foodstuff for infants As a result kwashiorkor and nutritional marasmus often occur and the chapter of the monograph entitled present status of nutritional disease among young children in the subtropics and tropics deals mostly with these two protein calorie malnutrition syndromes The author gives a short concentrated survey of the syndromes with special emphasis on the importance of early diagnosis and understanding of the miscellaneous factors in their etiology He also gives recommendations of treatment suitable for large hospitals as well as for rural hospitals In the chapters dealing with methods

f improving infant feeding in the subtropics and respect and prevention of nutritional diseases the author stresses the importance of clear-cut planning and the subsequent evaluation of the effectiveness and cost of the suggested programmes This is also a question of selection of programme priorities and is different from country to country The importance of not forgetting maternal nutrition is mentioned As a general recommendation for infant feeding the monograph repeatedly stresses the use of breast feeding alone for six months this is in fact regarded as the only way to survive in several parts of the world. From 6 months onwards mixed feeding is introduced with semisolid food that is locally available and acceptable and should be rich in vegetable and if possible animal protein given together with breastmilk thus saving the child from kwashiorkor when passing from the first safe 6 months of life to the age of 2 3 when it can have full adult diet To be able to change the pattern of infant feeding it is important to have nutritional education The last two chapters of the book are dealing with this problem To be able to overcome the difficulties a nutritional educator must know a lot about factors determining people's behaviour have good knowledge about local customs traditions and believes The author discusses all these problems in some detail and analyses the obstacles to success Among others is the dangerous influence of commercial advertising of milk food on mothers who have neither money nor education nor the kitchen facilities to handle such products If the fall for the temptation of being modern their child will often get an overdilute contaminated mixture low in nutrients and high in bacteria At the end of the book there are 15 appendices with short very practical advice on several topics dealt with in the text before There are also 26 illustrative photographs and about 700 references of which perhaps too many are old This book of a very experienced author gives a lot of facts and information about the life that is a reality for the majority of people in this world

Bo Balld n

D H Shmerling H Berger & A Prader (eds) *Symposium on Intestinal Absorption and Malabsorption* 15 pp illus S Karger Basel New York 1968 DM 50 — US $12 00

The proceedings of symposium on intestinal absorption and malabsorption in Zurich 1967 in honour of Prof Hottinger are published in this book As the contributors are wellknown biochemists paediatricians and gastroenterologists the contents are of good quality The book demonstrates clearly that the amount of information of normal and abnormal conditions in the gastrointestinal tract is rapidly increasing

The first part of the book deals with physiological problems such as the digestion and absorption of sugars and fats Margot Shiner presents beautiful electron micrographs in a chapter on the dynamic morphology of normal and abnormal small intestinal mucosa In spite of the fact that Prof Prader remarks in a wellcome address that the programme of the symposium is more restricted than the title indicates (e g coeliac disease and cystic fibrosis is not included) it is nevertheless surprising that nothing is said about the digestion and absorption of proteins under physiological conditions. This drawback of the book is however to some extent neutralized by two papers on amino acid transport in the second part of the book dealing with pathological conditions S Segal contributes with a paper on tissue transport defects of dibasic amino acids and Ch R Scriver on factors influencing uptake of amino acids in kidney and intestine Other papers in this part deal with methionine malabsorption mono- and disaccharide malabsorption chloride malabsorption and selective malabsorption of vitamin B_{12}

The last part of the book consists of three valuable chapters on general clinical problems normal and defective production of immunoglobulins in the intestinal tract by P A Crabbé and J F Heremans malignancy and steatorrhea by A F Read and effects of extensive intestinal resections in childhood by A W Wilkinson

Each paper is completed by a reference list and a discussion This book is of value for physicians not only paediatricians interested in the gastroenterological field [illegible]

Tor Lindberg

H M van Praag (ed) *Brain damage by inborn errors of metabolism* De Erven F Bohn N V Harlem, 1968 126 pp US $3 75

This society wants in an interdisciplinary setting to work with the problems concerning the relations between cerebral function and behavior—or disturbed function and disturbed behavior The present publication consists of a number of papers given at a symposium, held in Amsterdam 1967 on the subject of inborn metabolic disorders giving rise to brain lesions

The inborn errors of amino-acid metabolism are discussed in several chapters A list of the most important disorders known to day is given Aspects of pathogenesis diagnosis treatment and control are considered The subheadings are not treated separately however and there is some overlap of subject matter in different papers Nevertheless there is much valuable information in this part of the book

Another paper by W H H Tegelaers gives a qualified and detailed picture of the inborn errors of metabolism of the thyroid gland including biochemical histological and clinical features

According to the intentions of the society there is a survey of the intellectual psychological complications of brain damage by these disorders and a brief outline of the neurological examination of the infant is also presented.

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personal style with respect to writing but in spite of this the different parts have many features in common. Thus all main subjects are discussed both from theoretical and clinical point of view and several diagnostic laboratory procedures are included in detail which makes the book useful also as a manual in certain areas. The usefulness of the book is further extended by the large number of references to pertinent literature giving good guidance for those who want to go into more detailed studies of theoretical and/or clinical problems. The balance between theoretical aspects on one hand and clinical and practical on the other is as a rule adequate and in several cases these various aspects are discussed in relation to each other in a way making the presentation medically entertaining. Most chapters also contain pictures and figures which are most representative and clearly demonstrate that the authors combine theoretical knowledge with practical and clinical experience within their respective fields. It should also be noted that references to literature are most up to date.

This book definitely has its place within the paediatric literature and it can be warmly recommended both to the clinical paediatricians and to scientists within this field. In addition the book should be most useful also to doctors in other fields of medicine in serving as an introduction to clinical endocrinology on the whole.

Bernt Hokfelt and Karl Olof Nilsson

L. Diethelm, O. Olsson, F. Strnad, H. Vieten & A. Zuppinger (eds.) *Handbuch der medizinischen Radiologie*. Band X. Teil 1. H. Vieten (ed.): *Roentgendiagnostik des Herzens und der Gefässe*. 789 pp., illus. Springer Verlag, Berlin, Heidelberg & New York 1969. DM 296.

This book gives in about 700 pages an extensive description of the roentgen diagnosis of the heart and blood vessels. The topics described in this volume are various techniques of examination, radiokymographic, the use of contrast media in cardiac catheterization and the physiology, physiopathology and radiology of heart function and hemodynamics. The illustrations are of high quality. The reference lists are up to date to 1965. The book is of course indispensable for radiologists but can also be recommended to paediatric clinics to be used as a book of reference.

Tor Lindberg

A. Benedikt und P. Czákó: *Zur Frage der Entwicklung von Kindern mit niedrigem Geburtsgewicht*. 167 pp. Akadémiai Kiadó, Budapest 1969. US $6.00.

This book discusses the development of children with low birthweight (less than 2500 g). The study is made on a material of more than 6000 children with low birthweight from Budapest. The first part of the book deals with the late mortality of premature children and points out the rôle of congenital malformation and birth trauma. The second part gives a detailed study of the growth of these children compared with a group of children with normal birthweight and measured by four parameters: body weight, length, chest and head circumference. The results are statistically analyzed and a great acceleration in all four parameters is found during the first year of life, mostly pronounced in children with the lowest birth weight. During the second and third year the growth is about the same as for children with normal birth weight. There is no difference between boys and girls but there is evidence that this rapid growth in part depends on the medical care.

There is a short reference list and an extra chapter about the methods of statistical analysis.

The text is in German and rather difficult to read with many tables and mathematic formulas. It could however be recommended to those studying the development of infants with small birthweight because it is a thorough study on a big material and gives a somewhat different aspect on this subject.

Ingrid Bjerre

The volume will best serve as an introduction to the field of inborn errors of metabolism its bipolar conception is stimulating Both pediatricians and psychologists psychiatrists will find it useful

Sture Sjoblad

A J Moss & F H Adams (eds) *Heart disease in infants children and adolescents* The Williams & Wilkins Co Baltimore 1968 1140 pp illus US $48 50

During the last decade the field of pediatric cardiology has expanded very rapidly This expansion has made the preparation of a comprehensive pediatric cardiology textbook very difficult for one or a few authors These difficulties have mostly been overcome by publishing a textbook with 75 authors writing in their respective fields

In addition to the description of various acquired and congenital heart diseases the book contains a section of general cardiology with chapters on several subjects such as Fetal circulation and alterations at birth Genetic aspects of congenital heart disease Electrocardiography etc Other sections contain chapters on special problems such as Systematic hypertension Congenital heart failure and surgical aspects of pediatric cardiology The book is somewhat heterogenous but most chapters are of high quality while some are excellent The illustrations on the whole are of good quality Some criticism can of course be made i e the omission of mentioning the use of β blocking agents in the treatment of cyanotic episodes in tetralogy of Fallot

The book is highly recommended to everyone interested in pediatric cardiology

Nils Rune Lundstrom

G Sauter (ed) *Kinderpsychiatrie in der Praxis* Pädiatrische Fortbildungskurse fur die Praxis Vol 9 S Karger Basel and New York 1968 114 pp DM 24 —

This booklet with its 9 authors in 108 pages gives short informative essays In comparison with the Scandinavian elementary textbooks (A Anell M Lomholt E Regner) it is less systematic and seems to have the ambition to deal with some practically essential topics only often giving priority to therapeutic aspects Some of the authors give concretion with the help of case reports not aiming at completeness but at demonstration of traits and trends and obvious psychodynamic mechanisms In spite of undisputable instructive value such a limited and condensed selection has its risks To such readers who do not have a rich psychiatric experience of their own it may give the impression of a parade of cases not often seen in reality

The short essays reflect varying attitudes to description of psychiatric disease H Stutte builds his survey of psychoses with regard to disease entities and processes genetic connections developmental stages and special constitutional deviations A Weber describes different neurotic patients mainly from dynamic aspects He also mentions different constitutional disposition to reactions of ambivalence with different risks for neurotic symtom formation

W Brettschart writes about moyens therapeutiques chez des enfants souffrant de lesions cerébrales mineures He gives an instructive and sophisticated short analyse of minimal cerebral lesions and their interaction with psychosocial environment

In a description of therapy to mentally retarded children the nestor in child psychiatry J Lutz gives his skilful apology for whole hearted work for the mentally retarded children and for their right to a humanizing cultivation also in the most desperate cases He gives his experience also of the handling of paternal reactions The main point in his view is the importance of Heilpadagogik No parallells are described to the positive experiences from several other countries of actual efforts to integrate many of the mentally retarded systematically into normal social life away from traditional institutions as much as possible also in towns and cities with regard to living facilities school forms and work forms

It may seem somewhat strange that it was necessary in this actual booklet to warn for the view of glutamic acid as brain food Several authors in the booklet characterize the substance as an effective stimulating and sometimes valuable agent to a passive mind In several countries the substance has for some years been abandoned and is regarded as ineffective for such use

Corboz gives practical hints about quite dramatic magic trics in his essay about medicine to children with deviant behaviour among other things In this connection the question arises to what extent the frequency of patients who dare ask their doctor why might vary in different European countries

This booklet is a complement to the small textbooks covering more of the whole child psychiatric field It gives references to child psychiatric literature written in German

Ingmar Lagergård

D Hubble (ed) *Paediatric Endocrinology* Blackwell Scientific Publ Oxford 1969 £7 10s

During the last two decades there has occurred a remarkable development within theoretical and clinical endocrinology This has stimulated Do das Hubble former professor of paediatrics at Birmingham University to initiate the present book aiming at fulfilling a well recognized need within paediatric endocrinology The book contains 10 chapters covering altogether 492 pages Two of these chapters have been written by professor Hubble personally the 8 remaining ones by nine specialists seven representing paediatrics one genetics and one gynecology The various authors have been allowed a great deal of

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FREQUENCY AND NATURE OF RELAPSES IN CHILDREN SUFFERING FROM THE MALABSORPTION SYNDROME WITH GLUTEN INTOLERANCE

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According to the literature (2) gluten intolerance appears to exist in several different forms A typical feature of gluten intolerance in coeliac disease is said to be the disappearance of symptoms and mucosal abnormalities when gluten is avoided and their recurrence when it is ingested again (1) However it is well known that clinical symptoms of the disease do not always reappear (7) and this has led to the concept of acquired or transient gluten intolerance (2) Investigations based on *intestinal* biopsy however indicate that these patients may undergo relapse without any clinical manifestations (8)

The clinical symptomatology of gluten intolerance has earlier been described by one of us (10) in a group of infants suffering from the malabsorption syndrome Because the duration of these clinical symptoms was found to be relatively short, it was assumed that most of these patients had transient coeliac disease and they were therefore placed on a gluten containing diet when they were found to tolerate it clinically

The purpose of the present follow up study including intestinal biopsy was to throw further light on the real nature of the gluten intolerance in these patients and to investigate the prognosis of such intolerance

MATERIAL AND METHODS

The series comprised 23 patients seen during the years 1962 1964 in whom the malabsorption syndrome with gluten intolerance was diagnosed during the first 2 years of life Of these 23 19 were consecutive and non selected cases which have already been presented by one of us (10) The diagnosis of the malabsorption syndrome was based on the *following* symptoms prolonged diarrhoea and/or failure to thrive combined with the finding of an abnormally large amount of faecal fat (more than 4 g/day) and/or abnormally low D-xylose urinary excretion (less than 15%) Gluten intolerance was indicated by a successful response to a gluten free diet within 4 weeks (disappearance of symptoms and gain in weight) Patients with other food intolerances (cow's milk) are not included in this series The principle of treatment was to keep the patients on a gluten free diet (wheat rye oats and barley excluded) only for as long as was necessary to avoid the reappearance of clinical symptoms Therefore after clinical improvement on the gluten free diet was observed re-introduction of gluten was tried sooner or later Re-introduction consisted of giving the patient a single dose of 100–200 ml of a wheat-(3%) cow's milk mixture and if no acute reaction was noted the patient was transferred to gluten-containing diet normal for his age *If* the patient did not tolerate gluten on this first occasion reintroduction was tried again later A follow up study including biopsy was performed on all patients later

The methods used for investigations are described

Aided by a grant from the Sigrid Juselius Foundation

ANNOUNCEMENT

SUGGESTIONS CONCERNING THE NOMENCLATURE OF BIRTH WEIGHT AND GESTATIONAL AGE

On Tuesday 7th April 1970 an informal Working Party consisting of Obstetricians and Paediatricians from thirteen European countries who were attending the Second European Congress of Perinatal Medicine met and (after discussion) agreed to make the following suggestions

(*a*) That there is a need to define the important groups of babies who present special clinical difficulties and have an increased perinatal mortality in simple words which straightforwardly reflect what can be measured namely birth weight and gestational age (thus excluding all words incorporating maturity which cannot be measured as applied to a whole individual)

(*b*) That this should make it possible to apply to the intra uterine phase of growth the same concepts as have long been applied to the study and definition of growth after birth

(*c*) That the essential parameter is the gestational age calculated from the menstrual dates (and so perhaps better called menstrual age) Where reliable menstrual information is not available and there is information about the date of ovulation this can be used by adding 14 days to calculate the gestational age This should then ideally be expressed in days but if weeks are preferred they should be completed weeks (so that 40 weeks = 280-286 days) in presenting tables and graphs both the days and the corresponding weeks should be stated If no information is available it was not felt possible in the present state of knowledge to recommend any of the more technical methods of estimating the gestational age for general use

(*d*) If it is considered useful (because of differences in mortality and morbidity) to group babies in terms of gestational age the three groups and definitions suggested are

(i) Pre term less than 259 days (37 weeks)
(ii) Term 259–293 days (37–41 weeks)
(iii) Post term 294 days (42 weeks) or more

There was a considerable body of feeling in favour of placing the limit between pre term and term 7 days (1 week) later but the arguments in favour of this were not thought to outweigh the fact that 259 days is the official WHO recommendation (Technical Report Series no 25 1950)

(*e*) That birth weight is of some practical value in itself and when it is the only information available horizontal dividing lines of the traditional type may be useful—e g 2 500 g or less = low birth weight and sub-division by 500 g intervals may also be useful within this low birth weight group Separate recording of babies with a birth weight of 1 000 g or less is in any case useful when making statistical comparisons

(*f*) The usefulness of birth weight is much increased by relating it to the gestational age and comparing the results with the known distribution in a defined population (e g a percentile chart relating birth weight to gestational age preferably sex specific and based upon the population from which the clinical material is drawn—but otherwise upon the best data available for purposes of comparison) The birth weight can then be expressed in appropriate phrases—e g below 5th percentile for boys Aberdeen 1965 (or light for-dates)

Members of the Working Party

Chairman Dr G A Neligan U K

Members Dr A Ballabriga Spain Professor H Beutnagel vice Professor H Wulf Germany Professor Bucci Italy Dr P M Dunn U K vice Professor N E Butler Professor H Ewerbeck Germany (*Monatsschrift fur Kinderheilkunde Zentralblatt fur Kinderheilkunde*) Dr B Friis Hansen Denmark (Member Ed Board of *Acta Paediatrica Scandinavica* Member Ed Board of *Pediatric Research*) Dr D Gairdner U K (Editor *Archives of Disease in Childhood*) Dr J Gentz vice Professor R Zetterstrom Sweden (Editor *Acta Paediatrica Scandinavica*) Professor P O Hubinont Belgium Professor P Karlberg Sweden Professor G J Kloosterman Holland Dr F Kubli Switzerland Professor A A Minkowski France (Editor *Biologia Neonatorum* Editor *Developmental Psychology*) Professor L S Prod hom Switzerland Dr G Rooth Sweden Professor E Saling Germany Professor C Sureau France (Editor in Chief *Gynecologie et Obstetrique* Member of the Editorial Board of the *International Journal of Obstetrics and Gynaecology*) Dr T Valaes Greece Professor J Horsky Czechoslovakia

Secretary Professor P J Huntingford U K (*Assistant Editor of the Journal of Obstetrics and Gynaecology of the British Commonwealth*)

Table 2 *Symptoms and laboratory data in the eight symptomatic relapsers at the time of the relapse*[a]

Diarrhoea	7/8
Vomiting	2/8
Failure to gain weight	8/8
Haemoglobin under 11 g/100 ml	3/5
Serum iron under 40 µg/100 ml	5/5
Fat excretion more than 4 g/day	1/4
D-xylose excretion less than 15%	1/4
Positive urinary FIGLU	4/4
Precipitins to cow's milk	3/4
Precipitins to gluten	4/4
Elevated serum IgA	3/4
Small intestinal mucosa	
— Subtotal villous atrophy	4/4

[a] Only four of the symptomatic relapsers could be studied by us before return to the elimination diet. The remaining four were studied later and in all the small intestinal mucosa was abnormal at the time of examination.

tion diet. During this period eight of the 23 patients underwent a clinically evident relapse whereas the other 15 continued to be symptom free. In all these eight relapsers the diagnosis was verified by finding an abnormal intestinal mucosa at the time of the relapse or later. All 15 asymptomatic children were also biopsied and in 14 an abnormal mucosa was found.

Symptomatic relapsers. Symptomatic relapse became apparent soon after reintroduction of wheat, the average time being 6 4 months after the return to a normal diet. As the data in Table 1 indicate, there was not much difference either in the initial clinical picture or in the course of the disease between the symptomatic and asymptomatic relapsers. When a test for gluten intolerance was made during the initial elimination diet period only 1 of these 8 patients reacted. The symptoms in relapses (Table 2) were the same as in the initial phase of the disease although milder. In 1 case a change in the growth pattern was the only symptom. The results of absorption tests, immunological examination and biopsy were also abnormal.

Clinically all relapsers responded well to reintroduction of the gluten free-diet. Three of these 8 patients were again treated only by a short course of the elimination diet and have since then been asymptomatic but with an abnormal mucosa. The other 5 patients are being treated continuously: the mucosa has shown complete normalisation in one (at 3 3 years) and improvement in three (at 2 4, 3 6 and 3 8 years), whereas in 1 patient comparison is impossible because the initial biopsy at the relapse did not succeed.

Asymptomatic relapses. As mentioned earlier, 14 patients were clinically asymptomatic and growing normally during the whole observation period, the small bowel mucosa being clearly abnormal in each case. The average time for which these 14 asymptomatic patients were observed after resumption of a normal diet was 3 7 years (range 1 9–6 5 years). The results of laboratory tests made on these patients in connection with the follow up study are presented in Table 3. Two of the patients had an abnormal D xylose excretion test and one of these also had an abnormally large amount of faecal fat. Immunological tests also gave abnormal results in some patients.

Two non selected asymptomatic patients were later placed on a gluten free diet again. One of these had initially only been treated for 1 5 months, being then asymptomatic for 2 2 years although having a flat mucosa; on a long term gluten free diet the mucosa was found to have become normal after 2 4 years. Rein

Table 3 *Data of the 14 asymptomatic relapsers at the last follow up examination on a normal gluten containing diet*

Weight above 16 percentile	11/14
Weight above 2 5 percentile	14/14
Height above 16 percentile	12/14
Height above 2 5 percentile	14/14
Normal bone age	6/6
Haemoglobin under 11 g/100 ml	4/14
Serum iron under 40 µg/100 ml	2/12
Fat excretion more than 4 g/day	1/10
D-xylose excretion less than 15%	2/12
Positive urinary FIGLU	0/9
Precipitins to cow's milk	6/11
Precipitins to gluten	3/11
Elevated serum IgA	3/11
Small intestinal mucosa	
— Subtotal villous atrophy	11/14
— Partial villous atrophy	3/14

All were within two standard deviations and distribution was normal.

Table 1 *Data about the course of the disease main initial symptoms and findings at the time of diagnosis in different groups in the study*

	All	Symptomatic relapsers	Asymptomatic relapsers	Recovered
Number of patients	23	8	14	1
Male/female	11/12	4/4	6/8	1/0
Age at onset of symptoms (mean and range) in months	6 5 (4–18)	7 8 (4–18)	6 0 (4–10)	4
Age at time of diagnosis (mean and range) in months	9 7 (5–24)	10 2 (5 5–24)	9 7 (5 0–18)	5
Duration of gluten containing diet before diagnosis (mean and range) in months	6 9 (1–19)	6 7 (2 3–19)	7 5 (? 5–14)	1
Duration of initial treatment with gluten free diet (mean and range) in months	9 7 (0 6–25)	11 8 (2–25)	7 5 (0 6–19)	24
Duration of gluten containing diet before relapse or before last examination (mean and range)	2 6 (0 16–6 5) years	6 4 (2–18) months	3 7 (1 9–6 5) years	4 6 years
Prolonged diarrhoea	21/23	7/8	13/14	1/1
Vomiting	20/23	6/8	13/14	1/1
Failure to gain weight	23/23	8/8	14/14	1/1
Weight under 2 5 percentile	17/23	6/8	10/14	1/1
Weight under 16 percentile	23/23	8/8	14/14	1/1
Enlarged abdomen	15/23	4/8	10/14	1/1
Haemoglobin under 10 g/100 ml	7/23	3/8	4/14	0/1
Serum iron under 40 μ/100 ml	15/21	5/8	9/12	1/1
Fat excretion more than 4 g/day	21/22	8/8	12/13	1/1
D xylose excretion less than 15	18/22	7/7	10/14	1/1
Positive urinary FIGLU	18/22	5/7	12/14	1/1
Precipitins to cow s milk	16/16	4/4	11/11	1/1
Precipitins to gluten	11/16	3/4	8/11	0/1
Elevated serum IgA	10/16	4/4	5/11	1/1
Small intestinal mucosa				
— Subtotal villous atrophy	12/13	3/3	9/10	0 0
— Partial villous atrophy	1/13	0/3	1/10	0 0

earlier (3 4 11) Bone age was estimated according to the method described by Tanner *et al* (9)

Description of the patients

The average age of the patients at the time of diagnosis was 9 7 months and the mean duration of symptoms before admission 3 2 months The main symptoms and signs as well as the results of the diagnostic laboratory tests are given in Table 1 During the first 12 months after the start of the diet reintroduction of gluten was tried in 21 cases whereas in 2 cases the first attempt was only made after 2 years treatment for reasons not dependent on the clinical picture In the above mentioned 21 cases reintroduction was successful in 14 patients whilst 7 patients gave a clinical reaction to gluten The second attempt at reintroduction was successful in all these 7 Altogether the first reintroduction was successful in 16 cases and the second in all the remaining 7 cases

The mean duration of the initial gluten free diet was 9 7 months (range 0 6–25 months) But this cannot be taken as the length of the period during which the patients were intolerant to gluten because the last moment when they were still sensitive to gluten is not known precisely

At the time of completion of the diet every patient was in good clinical condition Eight patients had reached a normal weight while the others were still catching up In all the 8 patients tested the absorption function was normalized No biopsy was performed at the time of transfer to the normal diet

RESULTS

General outlines The average length of the follow up period until the last examination or until the patients were placed permanently on a gluten free diet was 2 6 years (range 0 2–6 5 years) after completion of the initial elimina

no significant differences could be detected in the primary symptomatology, in the clinical severity of the disease, in the results of laboratory tests or in the response to elimination of gluten. As a matter of fact the duration of the diet was longer in the symptomatic relapsers, but it is difficult to evaluate the significance of this observation because the duration of the diet was often dependent on irrelevant factors. Most of the clinical relapses occurred within a short while after reintroduction of gluten, and patients who had been asymptomatic for one year seemed to remain permanently symptom free.

Is it possible to detect these asymptomatic relapsers without a biopsy? At least the tests used in this study failed to do so. The highest incidence of abnormal results was found in the immunological tests but even these were normal in some cases.

One might now ask whether this mucosal damage in the asymptomatic patients is really gluten induced. We believe this to be the case although the causal relationship has not been directly confirmed in this study. It has been shown in at least one of our asymptomatic patients, who has developed a normal mucosa when returned to a gluten free diet. Since the year 1964, all similar patients have been treated by us with long term gluten free diet, and with this latter group it has been possible to demonstrate convincingly that the mucosal lesion really is gluten induced. The results of this study will be reported in due course.

However, in the present series there was 1 patient who probably recovered completely. Clinically this patient was a typical case of coeliac disease, even showing clinical symptoms in a provocation test. Unfortunately the initial biopsy was unsuccessful and this means that we do not posses incontrovertible proof of the existence of transient coeliac disease. Nevertheless this case suggests that transient clinical intolerance to gluten does occur. Further information on similar cases with full series of biopsies are needed.

SUMMARY

This is the report of a follow up study of 23 children who in infancy suffered from the malabsorption syndrome with gluten intolerance. These patients had been treated with gluten elimination only for the short period during which they were clinically intolerant to gluten. Three patients tolerated gluten after elimination for as little as 2 months, but the mean duration of elimination was 9.7 months. During the observation period 8 patients developed symptomatic relapse, mostly soon after reintroduction of gluten into the diet, whereas 15 continued to develop normally and were asymptomatic. However, 14 of these 15 were found to have an abnormal intestinal mucosa.

In 3 cases the symptomatic relapses were treated by a further short period on an elimination diet, after which the patients showed normal development, although the mucosa remained abnormal. Five of the relapsers were treated with long term elimination and improvement of the intestinal mucosa was observed in all of them. The only way to detect the asymptomatic patients was by biopsy, because the absorption tests and immunological tests failed to reveal most of them.

The only patient who was completely normal in the follow up study after consuming normal gluten-containing food for 4.6 years was initially a typical case of coeliac disease, also reacting to gluten in a provocation test, but unfortunately the diagnosis had not been verified by an initial biopsy.

REFERENCES

1. Anderson, C. M.: Intestinal malabsorption in childhood. *Arch Dis Child 41*: 571, 1966.
2. Frazer, A. C.: *Malabsorption syndromes*. William Heinemann Medical Books Ltd. London 1968.
3. Immonen, P.: Levels of serum immunoglobulins gammaA, gammaG and gammaM in the malabsorption syndrome in children. *Ann Paediat Fenn 13*: 115, 1967.
4. Kuitunen, P.: Duodeno-jejunal histology in malabsorption syndrome in infants. *Ann Paediat Fenn 12*: 101, 1966.

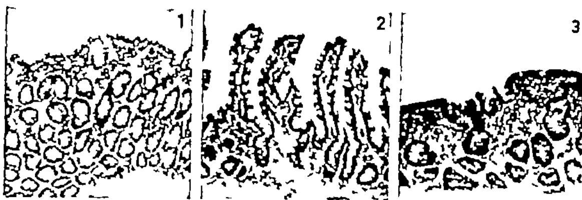


Fig 1 Small intestinal follow up biopsies of a boy on whom the diagnosis was made at the age of 7 months Primary biopsy showed clearly abnormal mucosa but the slides are technically poor The boy was kept for 1 5 months on gluten free diet after which he took normal food for 2 2 years and was doing well although biopsy (1) revealed a completely flat mucosa The boy was placed on gluten free diet and the mucosa normalized in 2 4 years (2) When he was exposed to gluten again for 7 months the villous atrophy recurred (3)

troduction of gluten after this caused deterioration of the mucosa again in 7 months (Fig 1) In the other asymptomatic patient the mucosa had not normalized when examined 2 3 years after elimination of gluten

Non relapsing patient Only 1 patient was completely normal in the follow up study This patient was admitted for the first time at the age of 5 months having had wheat in the diet for 1 month The symptoms were typical, faecal fat excretion 6 8 g/day and D xylose excretion test 14 % but no initial biopsy was taken The patient responded well to a gluten-free diet and a provocation test with gliadin was positive (vomiting and loss of weight) This patient was treated for 2 years with gluten elimination The follow up examination was performed 4 6 years after reintroduction of gluten At this point the patient was normal in every respect including absorptive function and mucosal morphology

DISCUSSION

In an earlier report concerning the initial phase of the disease in these patients special attention was drawn to *the relatively short duration* of the gluten intolerance in some cases It was suggested that these patients might have only a secondary intolerance to gluten The results of this follow-up study show that this was not the case but that all but apparently one of the patients were suffering from true coeliac disease

This report also shows as did for example, the results published by Sheldon (7) and Mortimer *et al* (5) that a great number of patients develop a clinically silent" state after the initial treatment It is surprising how short an elimination period may suppress the active clinical symptomatology 2 months or less was enough in 3 cases We feel that these patients of ours are unlikely to be exceptional in this respect, because they were unselected and consecutive The early age of our patients and the short duration of their disease before treatment may be one factor reducing the duration of the period of active gluten intolerance

These results also expose the fallacy of basing the diagnosis of coeliac disease on reappearance of the symptoms when gluten consumption is resumed because it is always possible that at the time when the provocation test was performed the period during which the patient would have shown clinical symptoms of sensitivity to gluten had already passed A biopsy is at present the only known way to *establish whether gluten intolerance is permanent*

Are there then primarily any differences between patients who exhibit symptomatic relapse and those who do not? In our series

no significant differences could be detected in the primary symptomatology in the clinical severity of the disease in the results of laboratory tests or in the response to elimination of gluten As a matter of fact the duration of the diet was longer in the symptomatic relapsers but it is difficult to evaluate the significance of this observation because the duration of the diet was often dependent on irrelevant factors Most of the clinical relapses occurred within a short while after reintroduction of gluten and patients who had been asymptomatic for one year seemed to remain permanently symptom free

Is it possible to detect these asymptomatic relapsers without a biopsy? At least the tests used in this study failed to do so The highest incidence of abnormal results was found in the immunological tests but even these were normal in some cases

One might now ask whether this mucosal damage in the asymptomatic patients is really gluten induced We believe this to be the case, although the causal relationship has not been directly confirmed in this study It has been shown in at least one of our asymptomatic patients who has developed a normal mucosa when returned to a gluten free diet Since the year 1964 all similar patients have been treated by us with long term gluten free diet and with this latter group it has been possible to demonstrate convincingly that the mucosal lesion really is gluten induced The results of this study will be reported in due course

However in the present series there was 1 patient who probably recovered completely Clinically this patient was a typical case of coeliac disease even showing clinical symptoms in a provocation test Unfortunately the *initial* biopsy was unsuccessful and this means that we do not posses incontrovertible proof of the existence of transient coeliac disease Nevertheless this case suggests that transient clinical intolerance to gluten does occur Further information on similar cases with full series of biopsies are needed

SUMMARY

This is the report of a follow up study of 23 children who in infancy suffered from the mal absorption syndrome with gluten intolerance These patients had been treated with gluten elimination only for the short period during which they were clinically intolerant to gluten Three patients tolerated gluten after elimination for as little as 2 months but the mean duration of elimination was 9 7 months During the observation period 8 patients developed symptomatic relapse mostly soon after reintroduction of gluten into the diet whereas 15 continued to develop normally and were asymptomatic However 14 of these 15 were found to have an abnormal intestinal mucosa

In 3 cases the symptomatic relapses were treated by a further short period on an elimination diet after which the patients showed normal development, although the mucosa remained abnormal Five of the relapsers were treated with long term elimination and improvement of the intestinal mucosa was observed in all of them The only way to detect the asymptomatic patients was by biopsy because the absorption tests and immunological tests failed to reveal most of them

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REFERENCES

1 Anderson C M Intestinal malabsorption in childhood *Arch Dis Child 41* 571 1966
2 Frazer A C *Malabsorption syndromes* William Heinemann Medical Books Ltd London 1968
3 Inmonen P Levels of serum immunoglobulins gammaA gammaG and gammaM in the mal absorption syndrome in children *Ann Paediat Fenn 13* 115 1967
4 Kuitunen, P Duodeno-jejunal histology in mal absorption syndrome in infants *Ann Paediat Fenn 12* 101 1966

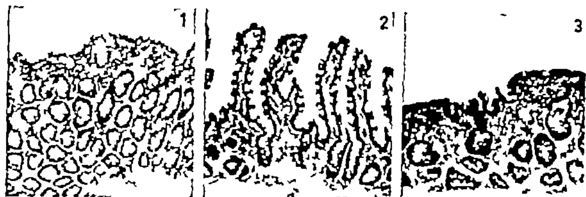


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DISCUSSION

In an earlier report concerning the initial phase of the disease in these patients special attention was drawn to the relatively short duration of the gluten intolerance in some cases It was suggested that these patients might have only a secondary intolerance to gluten The results of this follow up study show that this was not the case but that all but apparently one of the patients were suffering from true coeliac disease

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UNILATERAL NEUROLOGICAL DEFECT IN MYELOMENINGOCELE WITH NORMAL BLADDER FUNCTION

Report on Two Cases

N. O. ERICSSON, B. HELLSTRÖM, A. NERGARDH and U. RUDHE

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Various clinical reviews concerning myelomeningocele give the incidence of normal urinary control as between 3% (4) and 33, the latter figure originating from a somewhat selected series (3). With the cele in lumbosacral region the continence figures are generally low. The criteria of continence and particularly of normal bladder function vary. Sometimes the reports are based entirely on the history, sometimes bladder function is studied in a more detailed and objective manner. Only in recent years have conditions for the development of entirely intact bladder function begun to arise greater interest. Knowledge of these should constitute the basis of any clinical attempt to achieve continence. We shall here describe 2 cases of myelomeningocele which developed normal bladder function.

These 2 cases were among the last 50 consecutive patients with myelomeningocele in the lower thoracic or the lumbosacral areas who were referred for urological evaluation. This group was examined by a team of a neurologist, a radiologist and a urologist.

CASE REPORTS

Case 1. Boy with a lumbosacral myelomeningocele. At the age of $3^1/_2$ weeks transferred to the neurosurgical clinic. Report states that the boy moved his legs well and had anal sphincter function. No hydrocephalus. At operation nerve fibres were found passing distally into "indifferent" tissue in the lower right part of the sac. No diastematomyelia was present. The sac was excised. The postoperative course was uneventful.

Psychomotor development was normal until the age of 1 year when it was noted that the patient had difficulty in standing with support. His left leg was somewhat shorter than the right. With an orthopedic splint he began to walk and run although with a limp.

He developed control of urine and faeces at the age of 18 months but at 2 years of age began to develop encopresis and diurnal enuresis. At the same time and during the following years his home environment worsened considerably because his mother became paranoid.

The patient was referred for urological investigation at the age of 8 years. He showed complete control of urination and defecation. Normal stream on micturition. No leakage of urine on bladder expression. Normal urinalysis. Residual urine 5 ml. Normal renal function and cystometrogram.

Neurological investigation: Left leg 2 cm shorter than the right. On the left side decreased muscular strength in hip extensors, inward rotators and knee flexors, atrophy of musculature of the lower leg (Fig. 1), complete paralysis of the muscles of the foot and toes. Knee jerks normal bilaterally. Achilles and plantar reflexes absent on left side. Loss of cutaneous and temperature sensation corresponding to involvement of the first sacral segment and partial involvement of the fifth lumbar segment on the left side. Anocutaneous reflex normal. Normal anal and sphincter tone. X-ray showed defects of the arches of the 4th and 5th lumbar vertebrae. Intravenous urography and micturating urethrocystography were normal. No vesico-ureteral reflux was present.

Case 2. Girl with skin-covered lumbosacral myelomeningocele. Referred the first time on the

This work has been supported by a grant from Svenska Livförsäkringsbolags Nämnd för Medicinsk Forskning, Stockholm.

5 Mortimer P E Stewart J S Norman A P & Booth C C Follow up study of coeliac disease *Brit Med J* 3 7 1968
6 Rubin C E Celiac disease and idiopathic sprue Some reflections on reversibility gluten and the intestine *Gastroenterology* 39 260 1966
7 Sheldon W Celiac disease *Pediatrics* 23 132 1959
8 Shmerling D H An analysis of controlled relapses in gluten induced coeliac disease *Abst European Soc Ped Gastroenterology* 1968
9 Tanner J M Whitehouse R H & Healy M J R A new system for estimating the maturity of the hand and wrist with standards derived from 2600 healthy British children Part II The scoring system *International Children's Centre* Paris 1962
10 Visakorpi J K & Immonen P Intolerance to cow's milk and wheat gluten in the primary malabsorption syndrome in infancy *Acta Paediat Scand* 56 49 1967
11 Visakorpi J K Immonen P & Kuitunen P Malabsorption syndrome in childhood The occurrence of absorption defects and their clinical significance *Acta Paediat Scand* 56 1 1967

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moderate decrease in ability to move the foot and toes and unchanged left-sided atrophy of the calf muscles (Fig 2) and absent Achilles and plantar reflexes Decreased superficial sensation corresponding to the three lowest sacral segments dermatome on the left side as well as partially corresponding to the first sacral segment's dermatome on the right side Normal anal sphincter tone Normal intravenous pyelogram Micturating urethrocystography showed a bladder of normal capacity and demonstrated the previously noted asymmetry of the bladder neck The urethra was entirely normal and showed no sign of abnormal sphincter activity No reflux to upper urinary tract could be demonstrated

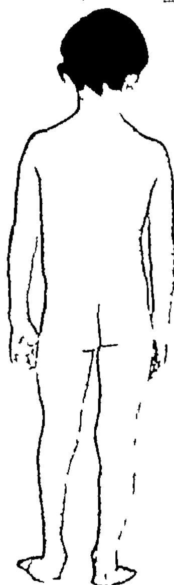
DISCUSSION

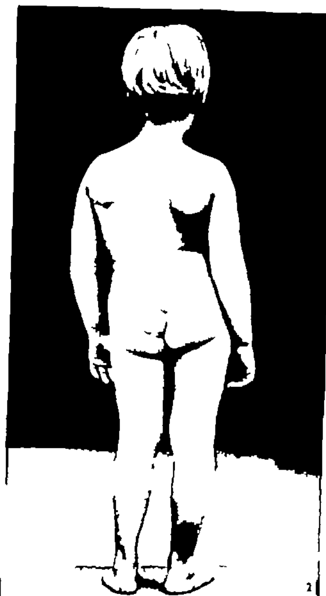
In both of these cases there was a cystic spina bifida in the lumbo-sacral region with clear evidence of loss of neurological function indicating myelomeningocele In both patients function loss corresponded to involvement of L_4–S_1 as the upper limit There was evidence of motor and sensory impairment as well as loss of reflexes but almost solely on the left side In both cases satisfactory bladder function was present as regards both detrusor and sphincter function Anal sphincter function was also normal Symptoms of enuresis and encopresis of psychogenic origin were present in the first patient

In case 2 there was uroradiological evidence of some changes in the bladder neck which showed an abnormal configuration during micturition characterized by right sided widening This remained a constant feature during subsequent examinations from the age of 1 week to 6 years It was noteworthy that this alteration primarily affected the right side where the loss of neurological function was hardly evident Moreover the left portion of the bladder neck repeatedly was found to be normal despite the presence of serious skeletal defects at the level of the sacral myelomeningocele on the same side

In most cases of myelomeningocele bladder function is altered Although exceptions have been noted the conditions necessary for retaining a certain level of bladder innervation have for the most part, seldom been discussed in the literature In this connection the level of the lesion is of some importance When the sac is situated in the cervical or upper thoracic spine neurological defects may be minimal and bladder function more or less normal (3) These locations however are relatively unusual (4) In cases of myelomeningocele involving the lower portion of the thoracic or thoraco-lumbar cord some function often remains in regions caudal to the level of the lesion—i e distal spasticity in the lower extremities or an intact anocutaneous reflex In such cases bladder alterations of upper motor neuron type with reflexmitrated partial or complete bladder-emptying may occur An intact reflex activity within the sacral cord can also lead to a hyperactive external sphincter which in turn may produce apparent partial continence In the long run however this is functionally unsatisfactory as "pseudo-continence" is accompanied by residual urine and involves a threat to kidney function

The importance of intact reflex activity in the 2nd to 4th segment of the sacral cord for partial detrusor function has recently been clarified by Stark (5) It seems possible that this reflex activity is more related to bladder function than the upper level of the lesion In his series there are some patients with normal bladder function and a number of them had diastematomyelia with unilateral lesion of the spinal cord Similar cases have also recently been described by Duckworth *et al* (2) who on surgical exploration found a hemimyelocele with preponderant unilateral dysplasia Moreover the majority of patients had for the most part, intact bladder function although a more detailed uroradiological investigation was not published Progressive scoliosis was an important feature accompanying the hemimyelocele This was not a prominent feature in our cases nor was an anatomical hemimyelocele found in the case operated upon Furthermore no sign of osseous diastematomyelia could be shown on roentgenograms Apart from the above mentioned studies there appears to be only one other re-





Figs 1 and 2 Patients presented in the text In both cases asymmetry of the neurological defect is appar ent with slight shortness of left leg and moderate atrophy of lower left leg

sixth day of life She was noted to have her legs flexed at hips but no definite neurological abnorma lities were noted

Roentgenological studies showed severe defects in the arches of the 4th to 5th lumbar vertebrae hypo plasia of the left aspects of all five sacral vertebrae and of the right aspect of L_4 as well as abnormal diastasis between the vertebral bodies and the rest of the vertebrae Intravenous pyelogram was normal Micturating urethrocystography showed a normal bladder During micturition there was reflux to the right ureter and kidney pelvis without dilatation The bladder neck was asymmetrically wider than nor mal on the right side A polyp was present in the proximal urethra which was otherwise normal

The urethral polyp was resected transurethrally The sacral tumour was not operated on and was con sidered to be a meningo or a myelomeningocele There was no hydrocephalus

Examinations at the ages of 4 and 9 months showed no change in the findings At 15 months the patient began to walk and it was noted that she limped The left leg was 2 cm shorter than the right On the left side there was a moderate degree of atrophy of the calf musculature and an absent Achilles reflex Bladder function was good Residual urine 4 ml On two occasions moderate bacteriuria but no clinical evidence of urinary infection Com plete control of both bladder and rectum Sensation of bladder and rectal filling remained intact Blad der neck showed the same asymmetrical shape as pre viously Bilateral vesico ureteral reflux now present

The last examination took place at the age of 6 years The patient was then completely continent Micturition normal No leakage of urine on bladder expression No residual urine Normal urinalysis without bacteriuria Normal renal function On the left side some decrease in strength of knee flexors

moderate decrease in ability to move the foot and toes and unchanged left-sided atrophy of the calf muscles (Fig. 2) and absent Achilles and plantar reflexes. Decreased superficial sensation corresponding to the three lowest sacral segment's dermatome on the left side as well as partially corresponding to the first sacral segment's dermatome on the right side. Normal anal sphincter tone. Normal intravenous pyelogram. Micturating urethrocystography showed a bladder of normal capacity and demonstrated the previously noted asymmetry of the bladder neck. The urethra was entirely normal and showed no sign of abnormal sphincter activity. *No reflux to upper urinary tract could be demonstrated.*

DISCUSSION

In both of these cases there was a cystic spina bifida in the lumbo-sacral region with clear evidence of loss of neurological function indicating myelomeningocele. In both patients function loss corresponded to involvement of L_4–S_1 as the upper limit. There was evidence of motor and sensory impairment as well as loss of reflexes but almost solely on the left side. In both cases satisfactory bladder function was present as regards both detrusor and sphincter function. Anal sphincter function was also normal. Symptoms of enuresis and encopresis of psychogenic origin were present in the first patient.

In case 2 there was uroradiological evidence of some changes in the bladder neck which showed an abnormal configuration during micturition characterized by right sided widening. This remained a constant feature during subsequent examinations from the age of 1 week to 6 years. It was noteworthy that this alteration primarily affected the right side where the loss of neurological function was hardly evident. Moreover the left portion of the bladder neck repeatedly was found to be normal despite the presence of serious skeletal defects at the level of the sacral myelomeningocele on the same side.

In most cases of myelomeningocele bladder function is altered. Although exceptions have been noted the conditions necessary for retaining a certain level of bladder innervation have for the most part seldom been discussed in the literature. In this connection the level of the lesion is of some importance. When the sac is situated in the cervical or upper thoracic spine neurological defects may be minimal and bladder function more or less normal (3). These locations however are relatively unusual (4). In cases of myelomeningocele involving the lower portion of the thoracic or thoraco-lumbar cord some function often remains in regions caudal to the level of the lesion—i.e. distal spasticity in the lower extremities or an intact anocutaneous reflex. In such cases bladder alterations of "upper motor neuron type" with reflexmitated partial or complete bladder-emptying may occur. An intact reflex activity within the sacral cord can also lead to a hyperactive external sphincter which in turn may produce apparent partial continence. In the long run however this is functionally unsatisfactory as "pseudo-continence" is accompanied by residual urine and involves a threat to kidney function.

The importance of intact reflex activity in the 2nd to 4th segment of the sacral cord for partial detrusor function has recently been clarified by Stark (5). It seems possible that this reflex activity is more related to bladder function than the upper level of the lesion. In his series there are some patients with normal bladder function and a number of them had diastematomyelia with unilateral lesion of the spinal cord. Similar cases have also recently been described by Duckworth *et al.* (2) who on surgical exploration found a hemimyelocele with preponderant unilateral dysplasia. Moreover the majority of patients had for the most part, intact bladder function although a more detailed uroradiological investigation was not published. Progressive scoliosis was an important feature accompanying the hemimyelocele. This was not a prominent feature in our cases nor was an anatomical hemimyelocele found in the case operated upon. Furthermore no sign of osseous diastematomyelia could be shown on roentgenograms. Apart from the above mentioned studies there appears to be only one other re-

port describing a few cases of asymmetrical innervation all of which had normal sphincter control (1)

The cases here described confirm the view that when myelodysplasia with cystic spina bifida primarily causes unilateral neurological disturbance, function may be sufficiently retained within the 2nd to 4th sacral segment to permit entirely normal bladder and anal sphincter activity

The true incidence of such asymmetrical defects cannot be determined from our series Less evident asymmetries, particularly those concerning sensory disturbances are common Frequently there is a difference between both sides as regards the extent of the lesions, which may involve one or two segment levels

More account should be taken of the minority of cases with myelomeningocele which have partial or completely normal bladder function Increased knowledge of such cases can further clarify studies of incontinence This in turn, can lead to a more rational approach to the conquest of this very troublesome social handicap

SUMMARY

Two cases of myelomeningocele with symptoms of unilateral disturbance and with normal bladder function are discussed

REFERENCES

1 Doran P A & Guthkelch A N Studies in spina bifida Part IV The frequency and extent of paralysis *J Neurol Neurosurg Psychiat 26* 545 1963
2 Duckworth T Sharrard W J Lister J & Seymour N Hemimyelocele *Develop Med Child Neurol Suppl 16 69 1968*
3 Eckstein H B Urinary control in children with myelomeningocele *Brit J Urol 40* 191 1968
4 Smith, E D *Spina bifida and the total care of spinal myelomeningocele* Charles C Thomas Springfield Ill 1965
5 Stark G Pathophysiology of the bladder in myelomeningocele and its correlation with the neurological picture *Develop Med Child Neurol* Suppl *16 76* 1968

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FACTORS PROMOTING URINARY AND ANAL CONTINENCE IN CHILDREN WITH MYELOMENINGOCELE

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In a previous study (5) 2 cases of myelomeningocele with normal bladder function were described In these patients the clinical picture was characterised by unilateral disturbance of neurological function They were included in a recent series of 50 cases investigated neurologically radiologically and urologically The degree of severity and the type of incontinence or partial continence varied In the literature diverging opinions are found concerning the relationship between remaining bladder function and the neurological findings

In this study an attempt has been made using ordinary clinical methods to determine the conditions for acquisition of partial bladder and anal continence The aim has been to select suitable cases which might respond to bladder training either partially or completely and thus permitting the social rehabilitation of the patient The literature on this subject is scanty

MATERIAL

The case material consists of 24 boys and 26 girls who were referred for urological investigation The majority were admitted to the hospital on more than one occasion and examined regularly twice a year Some have been studied from the newborn period At the time of the last examination their ages ranged from 2 to 16 years The degree of continence has been determined from the history and from the clinical observations as regards habitual and maximal dry periods

The micturition history has included information on the patient's ability to experience the sensation of bladder filling and a desire to void as well as the sensations involved in voiding The ability to initiate micturition has been noted and whether this has occurred with or without increasing the intra-abdominal pressure The type of stream and the patient's capacity to voluntarily interrupt micturition have also been recorded During hospitalisation micturition has been studied in detail and compared with the information given in the history Analyses has invariably included an investigation of the response of the bladder to expression measurement of residual urine after spontaneous micturition or expression urine analyses, including quantitative bacteriological culture urine osmolality and serum creatinine or blood urea nitrogen. In some cases cystometry and kidney function tests were carried out. Intravenous urography and micturition urethrocystography were regularly performed

Anal function was evaluated on the basis of the history and hospital records as regards continence and its relationship to the consistence of stools The patient's ability to experience the desire to defecate on filling of the ampulla was noted The physical examination included inspection of the anal region evaluation of anal sphincter function by palpation and investigation of the anocutaneous reflex Roentgenologic studies of defecation were performed in 9 cases The rectum was filled with thick barium contrast medium and frontal and lateral views were taken In several cases the patient's ability to voluntarily elevate his pelvic floor was studied

The neurological evaluation included demonstration of motor and sensory loss as well as cutaneous and tendon reflexes. On the basis of these findings the patients were grouped according to the neurological level present of the various lesions Special attention was paid to the occurrence of preserved

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functions caudal to the level of the lesion Neurological classification according to the upper level of the functional lesion resulted in the following

Group 1 (low thoracic) Complete paraplegia was found sometimes accompanied by loss of strength in the trunk and particularly the abdominal muscles There was generally a total loss of sensations corresponding to the level of the motor lesion or with a somewhat lower cranial level In some cases there was evidence of spasticity accompanied by ankle clonus and positive Babinski The anocutaneous reflex was persistent in some of these cases

Group 2 (high lumbar) Subtotal paraparesis was present and in some cases accompanied by residual function in the hip flexors and adductors Loss of sensation corresponded to the level of the motor lesion but at times to a somewhat lower cranial level In most cases reflexes were entirely absent in the lower extremities Spasticity and/or persistent anocutaneous reflex were occasionally present

Group 3 (lower lumbar) The function in hip flexors adductors and knee extensors were well maintained Sensation corresponded to the level of the motor lesion or to a somewhat lower cranial level Normal or slightly decreased patellar reflexes absent Achilles and plantar reflexes were noted Spasticity did not occur In some cases anocutaneous reflex persisted

Group 4 (high sacral) Motor losses included impaired function of hip extensors abductors and knee flexors as well as considerable decrease in ability to move the feet Decreased sensation corresponding to the level of the motor lesion or to a somewhat lower cranial level was observed Achilles and plantar reflexes were absent Neither spasticity nor anocutaneous reflex were present

Group 5 (low sacral) No motor loss was observed Sensory loss corresponded to the lowest sacral segment Tendon reflexes were intact No spasticity was recorded The anocutaneous reflex was not present

Detailed analysis of radiological data concerning the urinary tract and correlation with the urological and neurological findings will be published separately

RESULTS

Partial urinary continence Of the 50 patients examined, 2 were completely continent and were able to control micturition Both belonged to the upper sacral group from a neurological point of view but had unilateral functional loss Of the remaining 48 patients four were less than 2 years of age at the time of the last examination and in 8 cases the Bricker procedure was used for urinary diversion For this reason it is not possible to make a final judgement concerning continence in those 12 cases

Table 1 *Groups of urinary incontinence and neurological levels*

	Dribbling incontinence	Dry periods of more than 1 hour s duration
Low thoracic	1	1
High lumbar	3	6
Low lumbar	0	3
High sacral	7	9
Low sacral	2	4
Total	13	23

The remaining 36 patients were divided into two groups according to the degree of continence In the first group 13 patients had continuous dribbling incontinence or dry periods of less than 1 hour s duration In the second group 23 patients had dry periods of at least 1 hour and often 2 to 3 hours daily The distribution of the levels of the neurological defects appeared to be similar for both groups None of these patients had been given bladder training (Table 1)

Eight boys and 5 girls had dribbling incontinence while 9 boys and 14 girls had partial continence for more than 1 hour

Of those patients who later had a urinary diversion six were dribbling incontinent and one had dry periods of more than 1 hour The eighth patient was operated on before the age of two

Table 2 *Residual bladder function and groups of incontinence*

De = Detrusor function Sph = sphincter function Se = bladder sensation

Residual function	Dribbling incontinence	Dry periods of more than 1 hour	Total
De + Sph + Se	0	2	2
De + Sph	0	4	4
De + Se	0	4	4
De	1	5	6
Sph	0	0	0
Se	1	4	5
No residual function	11	2	13
No data	0	2	2
Total	13	23	36

In Table 2 both groups have been compared as regards residual function in the detrusor as well as sphincter function and bladder sensation At least partial detrusor function was considered present when the patient was able to urinate with a stream without increasing the intra abdominal pressure The sphincters were considered to possess some function if the bladder was not expressible and/or the patient was able to voluntarily interrupt micturition A certain degree of retained sensation during micturition was reflected in the patient's ability to be at least partly aware of bladder filling

In the completely dribbling incontinent group only two of 13 patients (15) had some detrusor function or sense of bladder filling In the group with longer dry periods corresponding values were 19 of 21 of those patients who could be evaluated (90) Detrusor function alone or combined with other types of residual function was considerably more common in the partially continent group Table 2 also shows that persistent sphincter function was not a necessary prerequisite for partial continence Moreover retained bladder sensation alone was probably more common in the partially continent group A functioning sphincter was invariably associated with signs of detrusor activity and bladder sensation This would indicate that residual detrusor function may be the chief requirement for dry periods of more than one hour's duration The partial continence often evolved about the age of 4 years

Control of defecation For 49 patients satisfactory data of anal function were available The cases were divided into three groups In the first group the patients were continent regardless of whether the stools were formed or not. In the second group (pseudo-continent) the patients were almost regularly continent if the stools were not loose In the third group the patients were incontinent even when the stools were of normal consistency In Table 3 the three groups are classified according to the level of the neurological lesion

Table 3 *Groups of anal function and neurological levels*

	Continent	Pseudo-continent	Incontinent
Low thoracic	1	2	3
High lumbar	1	4	4
Low lumbar	1	2	2
High sacral	5	9	8
Low sacral	1	5	1
Total	9	22	18

In this series it was not possible to show any definite relationship between the level of the neurological lesion and the degree of continence More detailed analysis was made in these three groups as regards sphincter tone anocutaneous reflex and sensations of ampulla filling or the need to defecate The results are shown in Table 4 In some cases the patients were too young to be able to adequately describe ampulla sensation

Normal sphincter tone had a favourable prognosis as had partial sphincter tone at least to some extent In the completely incontinent group no patient had sphincter tone The

Table 4 *Different parameters of anal function*

Degree of continence	Sphincter tone			Anocutaneous reflex			Ampulla sensation		
	Normal	Weak	Absent	Normal	Weak	Absent	Present	Absent	No information
Continent (9 cases)	3	6	0	3	2	4	9	0	0
Pseudocontinent (22 cases)	0	3	19	0	1	21	1	14	7
Incontinent (18 cases)	0	0	18	3	0	15	2	8	8

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Roentgen findings were normal in all respects in four cases with fecal continence. Of those classified as pseudo-continent 2 patients showed normal findings while one demonstrated a slightly lowered pelvic floor. Both patients with incontinence (aged 1 and 9 years) had a depressed pelvic floor and were unable to retain the contrast medium injected into the rectum and to close the anal canal and the external sphincter (Fig. 1). The latter patient could not voluntarily elevate the pelvic floor.

DISCUSSION

The incidence of urinary incontinence in unselected cases of myelomeningocele is high. Sacs located high in the cervical and upper thoracic spines often are compatible with entirely satisfactory bladder function (2, 4, 6). This is also true of hemimyelocele (3, 7) as well as other celes in the sacral region with unilateral functional defects (5). These localisations and types, however, are less common. In the more common lumbosacral lesions with relatively symmetrical functional defects, the incidence of incontinence is often stated to be as high as 97 % (6). From a clinical point of view it is possible to distinguish between the incontinent patients who are always wet from those who have shorter or longer dry periods. In the latter group, dry periods of as much as a couple of hours duration can at times be socially acceptable.

In the present series the group with dry periods of one or more hours duration is larger than the group which is permanently wet. Partial continence almost always requires some residual function of the detrusor, as judged by the ability to micturate voluntarily with a stream without increasing the intra-abdominal pressure. Residual function of the sphincter was less significant, and the same applied to afferent sensations in the bladder and urethra. This type of partial and voluntarily controlled micturition is different from the trigger released reflex type of bladder-emptying present in cases of high spinal-cord injury. In cases of myelomeningocele lesions of both upper and lower motor neurons may occur. However, flaccid paralysis is usually dominant and it seldom happens that enough function of the sacral cord is preserved to permit reflex emptying of the bladder. Moreover, neither in this nor in other available materials was it possible to show that minor differences of the upper level of the lesion had any decisive importance for the achievement of partial continence. On the other hand, partially maintained motor functions and reflexes innervated from the 2nd–5th sacral segments showed a positive correlation to residual detrusor function accompanied by partial continence (1, 3).

Smith (6) stated that the presence of large quantities of residual urine and difficulty in expressing the bladder were characteristic of subjects presenting with partial continence. The conclusion was drawn that this was related to the degree of resistance to flow in the urethra, depending inter alia on sphincter function.

The present study is based on simple clinical examinations. It is likely that more detailed studies including, for example, simultaneous pressure flow measurements can provide more precise information regarding bladder function and better evaluation of the prospects for successful bladder training and future continence. Using a modified method of cystometry Cooper in 1968 found normal detrusor activity in 35 % of the cases studied and most of those patients were expected to become continent after bladder training. It should be noted, however, that 15 to 20 % of the subjects showing no signs of detrusor activity became continent on training.

Eckstein (4) considered that if continence had not been achieved by the age of 3 it was unlikely to be attained later. The relatively high incidence of continent patients in his series (33 %) corresponds to an unusually large number of cases with celes in the cervical or upper thoracic regions.

In clinical reports of cases of myelomeningocele comparatively little attention has been de







Fig 1 Male 9 years—Rectum filled with semisolid barium contrast (a and b) Marked descent of the atonic pelvic floor with caudal displacement of the rectum partial obliteration of the wedge of the levator ani muscle immediately posterior to the rectum comprising the puborectalis component and funnelling of the anal canal which is incompletely closed (c) The cooperative patient is unable to elevate the pelvic floor

sensation of fullness of the ampulla or the desire to defecate showed a strong positive correlation to the subsequent development of continence although exceptions occurred All patients who had both ampulla sensation and some degree of sphincter tone were completely continent The anocutaneous reflex showed a poor correlation to the degree of continence

It should be noted that patients who developed a satisfactory degree of anal continence did so when relatively young many at 2–3 years of age In the second group pseudo continence developed around 7 years of age Several of the completely incontinent patients were quite young and classified in group 3 for the present

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RESPIRATORY PATHOLOGY IN THE IMMEDIATE POSTNATAL PERIOD

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Respiratory disorders in the immediate postnatal period correspond to a clinical syndrome which represents diverse anatomical disorders of the lung the majority of which may only be differentiated and classified through a very close histological examination It is not possible to establish a strict correlation between the clinical symptoms and the pulmonary anatomical findings

Our work is mainly orientated on the incidence of severe forms of respiratory distress classified according to the Silverman Andersen retraction score Relationship with findings in a large series of post mortem examinations have been obtained in order to establish the frequency of pulmonary involvement according to age and weight

MATERIAL AND METHODS

The entire tabulated material has been personally observed in this Children Hospital and in the attached Maternity unit during a 27 months period (March 1966 to May 1968)

In a total of 32 953 observed infants 31 520 were full terms and 1 433 prematures In the full term group 31 330 were born in the Maternity Hospital and 190 in different clinics of the city and admitted afterwards to our Unit for Pathological New borns

In the group of 1 433 prematures 933 were born in the Maternity Hospital and 500 were admitted to our Unit for Prematures during the first day of life proceeding from local clinics

Apgar index taken 1 min after birth was studied in 20 000 full term infants proceeding from the group of 31 330 born in the Maternity Hospital and also in the group of 933 prematures Silverman & Andersen's (14) retraction score at 3 hours of life was studied in all 32 953 cases.

For the estimation of incidence of respiratory distress we have included all cases showing a retraction score in stadia I or II at 3 hours of life Cases in stadia 0 at the moment of the first examination but showing during the following 72 hours a clinical evolution to stadia I or II were also tabulated

Variations of retraction score after 72 hours of life were not considered in our statistics because acquired infections after birth may play a role in these cases

Microscopic examination was performed in all organs with special emphasis to the lungs

The pathological material has been studied by the same team according to a previously established criteria however if there is a certain personal influence in the selection—as happens in all anatomopathological classifications—this is extended uniformly over all the material The lung was classified according to the most extensive and important histological feature that could condition the death. The material was divided under the following headings

(*a*) Hyaline membrane the undoubtable typical forms are included. When material was observed only suggesting the diagnosis of membrane" the case was classified according to the other existing lesions

(*b*) Atelectasis without hyaline membrane These cases have been included because at times they are the only pulmonary alterations present, without being considered as the primary cause of death Concurrently the existence of another more extrapulmonary cause was quoted.

(*c*) Immaturity this has been classified in 4 grades. Grade III corresponds to the maximum degree of immaturity It refers to the lung in canalicular state with the presence of canaliculi and thick septa with collagenized stromal tissue and the capillary vessels removed from the alveolar air spaces Stadium I

voted to the problem of anal continence It is generally stated that in most cases a socially acceptable continence exists provided the patient is kept constipated Here, of course, diet and a strict defecation regime may play an important role The extent of paresis of the extremities may indirectly affect the prospects of success for such a programme Detailed analysis of the neurological conditions favouring anal continence have not been published In the present material as in earlier published studies evidence of anal sphincter function was more common when the cele was located in the lower part of the thoracic cord or the upper part of the lumbar cord In our material, the patient's ability to experience the fullness of the ampulla and an urge to defecation was of great significance These afferent functions appear to have no direct relationship to the level of the lesion of the cele They were to a large extent positively correlated to complete anal continence, regardless of the consistency of stools

It is apparent that the present knowledge of the factors of significance for bladder and anal continence are only incompletely understood and that further detailed clinical and roentgenologic studies of cases of myelomeningoceles might be of help in designing further measures to promote continence

SUMMARY

Patients with myelomeningocele are due to present with varying degrees of urinary and anal incontinence The present study represents an attempt to elucidate the factors of significance for selection of patients suitable for training to achieve socially acceptable continence It would seem that an active detrusor is the chief factor determining urinary continence, whereas sphincter function and bladder sensation appear less significant As regards anal continence sensation in the ampulla seems most important

REFERENCES

1 Cooper D G Bladder studies in children with neurogenic incontinence With comments on the place of pelvic floor stimulation. *Brit J Urol 40* 157 1968
2 Doran P A & Guthkelch A N Studies in spina bifida Part IV The frequency and extent of paralysis *J Neurol Neurosurg Psychiat 26* 545 1963
3 Duckworth T Sharrard W J Lister J & Seymour N Hemimyelocele *Develop Med Child Neurol* Suppl *16* 69 1968
4 Eckstein H B Urinary control in children with myelomeningocele *Brit J Urol 40* 191 1968
5 Ericsson N O Hellstrom B Nergårdh A & Rudhe U Unilateral neurological defect in myelomeningocele with normal bladder function *Acta Pædiat Scand 59* 487 1970
6 Smith E D *Spina bifida and the total care of spinal myelomeningocele* Charles C Thomas Springfield Ill 1965
7 Stark G Pathophysiology of the bladder in myelomeningocele and its correlation with the neurological picture *Develop Med Child Neurol Suppl 16* 76 1968

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Key words Myelomeningocele urinary continence anal continence

Table 3 *Distribution of 700 autopsic cases according to age*

	Period					
	0–24 h	24–48 h	48 h–5 days	5–10 days	10–30 days	Total
Prematures 500–1 250 g	66	24	26	11	5	132
Prematures 1,251–2,500 g	106	52	59	39	51	307
Full term newborns > than 2 500 g	81	47	62	24	47	261
						700

F T – Full term P – Prematures

Statistical treatment of data of this group and the main pathological findings were made with the following formula

$$Qe = \frac{a1 + a2}{+\ ?} \qquad S^2d = Qe(1 - Qe)\left(\frac{1}{n1} + \frac{1}{n2}\right)$$

$$t = \frac{q1 - q2}{sd}$$

RESULTS

A *Study of the Apgar index* Results concerning 1 min Apgar index (2 3) performed in 20 000 full terms and 933 prematures have been compared with the results obtained in various previous studies and the distribution of neonatal mortality classified by scores The mortality rate is related to the period 0–30 days of life and the frequency and mortality of each score is quoted in Table 1

B *Study of the frequency of respiratory distress in full term and premature infants* The study of the frequency of the appearance of respiratory distress has been based on a batch of 31 330 observed full term births (normal vaginal delivery 89 4%—forceps 3 09 — Caesarian section 2 9%—bridge delivery 2 6 —vacuum 1 6 —Version 0 1%) classified according to the Silverman score and the results were as follows Full term births observed 31 330—number of respiratory distress

Table 4 *Distribution of pulmonary findings in 700 infants (439 prematures and 261 full terms) dead in the period 0–30 days of life*

	0–24 h		24–48 h		48 h–5 days		Total 0–5 days		5–10 days		10–30 days		Total ~ 5–30 days	
	FT	P	FT	P	FT	P	FT	P	FT	P	FT	P	FT	P
Total number	81	172	47	76	62	85	27 1*	47 5*	24	50	47	56	10 1	15 1
Hyaline membrane	12	72	12	37	8	16	17 0	37 4	0	2	0	0	0	1 8
Atelectasis without H M ᶜ	13	16	6	5	7	7	13 6	8 4	0	4	1	1	1 4	4 7
Immaturity[d]	0	30	0	9	0	8	0	14 1	0	2	0	0	0	1 8
Aspiration	8	4	6	3	6	6	10 5	3 9	2	2	0	2	2 8	3 7
Haemorrhages[d]	15	13	4	7	12	17	16 3	11 1	7	12	2	7	12 6	17 9
Pulmonary hypoplasia	13	8	0	0	0	0	6 8	2 4	1	0	0	0	1 4	0
Pneumonia	12	18	8	9	18	28	20 0	16 5	9	27	31	35	56 0	58 4
Listeriosis	1	6	1	1	1	1	1 5	2.4	0	0	1	0	1 4	0
Absence of pulmonary participation	7	5	10	5	10	2	14 2	3 6	5	1	12	11	24 0	11 3

The number of severe malformed cases in the whole of this series was 26/190 – 13 6 %

[a] The number of severe malformed cases in the whole of the series of prematures was 36/333 – 10 8 %

The number of gross congenital abnormalities in the whole of these series was 13/106 – 12.2 % and 6/71 – 8 4 % respectively

[d] In 31 prematures belonging to these three groups brain damage was demonstrated which could be the main cause of death and in 70 cases of the full term series

Table 1 *Distribution of neonatal mortality by scores in various studies*

Author	Total no of infants	Total mortality ( )	Total full term	Total prematures	Apgar index		
					Score 10–7 ( )	*Score 6–4* ( )	*Score 3* ( )
Apgar & James 1962	27 715	1 7	—	—	F 79 3 M 0 4	F 13 3 M 2 2	F 74 M 21
Drage *et al* 1964	17 221	1 4	—	—	F 78 9 M 0 4	F 14 5 M 2 1	F 67 M 115
Ikonen 1967	11 855	1 2	—	—	F 96 9 M 0 5	F 1 9 M 13 2	F 12 M 34
This study	20 933	1 4	—	—	F 95 5 M 0 6	F 2 89 M 12 6	F 15 77 28 4
			20 000	—	F 96 4 M 0 15	F 2 39 M 7 7	F 11 M 19 2
			—	933	F 76 8 M 13 3	F 13 6 M 33 5	F 94 M 53 4

F = Frequency M = Mortality

shows zones with discreet signs of immaturity slight alveolar development and cuboid type epithelial alveolar investment Grade II represents the intermediate forms between Grades III and I Grade 0 is the normal mature lung

(*d*) Aspiration In the 0–5 days group only the massive forms of amniotic or meconial aspiration have been considered The 5–30 day group includes fundamentally alimentary aspiration at times with pulmonary granulomatous reaction and aspiration of gastric juices frequently associated with a necrotizing pneumonia

(*e*) Haemorrhages The massive forms of haemorrhage have been included which affect uniformly two or more complete lobes and large diffuse intraalveolar and interstitial submassive haemorrhages considering the examination of pulmonary fragments from different zones

(*f*) Pulmonary hypoplasia These represent the cases which presented a total pulmonary mass weighing less than 50 of the normal weight for the age and body weight independently of the degree of pulmonary maturity All the cases included in this group were associated with severe malformations as diaphragmatic hernia and or cystic renal dysplasia or renal agenesia

(*g*) Pneumonia In the group of deaths occurring from 0–48 hours there are included the forms of intrauterine pneumonia with very abundant infiltration of leukocytes without the presence of fibrine without necrosis and with or without pathogens Beyond 48 hours all the types of pneumonia are catalogued as acquired pneumonias Included in th group of acquired pneumonias are (*a*) necrosis pr ducing or haemorrhagic granulocytary pneumon (*b*) interstitial pneumonia (*c*) interstitial with gia cells (*d*) pneumonias accompanying aspiration wi various degrees of desquamative alveolitis (*e*) pneu monia with presence of candida albicans

(*h*) Listeriosis There are included the pulmona forms with disseminated pulmonary granulomatos and identification of listerias in the lung tissue

(*i*) Absence of pulmonary participation These in clude the cases in which there were no ostensibl findings in the lung and the main cause of dea was removed from the lung Nevertheless it is fre quently possible to find included in this group minimum pulmonary histological participation in th form of a minimum granulocytary exudation r duced to the presence of 3 to 4 leukocytes per a veolar air space without uniform distribution A though a very high number of these deaths had pre sented asphyxia at the time of birth we have no classified the anoxia isolatedly among the pulmonar findings and these cases are included within the grou of the respective alterations they presented

Incidence of respiratory distress in 406 sets of twin was also studied These twins were taken in the grou of infants born in the Maternity Hospital

Table 2 *Incidence and mortality in severe respiratory distress in prematures*

	Total numbers	Very severe distress	Frequency ( )	Death cases	Mortality ( )
Prematures 500–1 250 g	112	40	35 7	38	95
Prematures 1 251–2 500 g	1 321	49	3 7	44	89 9

respiratory distress in a total of 31 330 children born in the Maternity Hospital 16 313 were males and 15 017 females giving a ratio of 1 male to 0 920 female The number of respiratory distress cases was 742 of which 432 males and 310 females giving a ratio of 1 male/0 717 female Thus the incidence of respiratory distress has been higher in the males with respect to the normal ratio of male/female births the difference being significant

C *Incidence severity and mortality rate of respiratory distress in twins with reference to sex* Muirr & Futrakul (13) have recently reported a higher incidence of respiratory distress in males over females especially in twins of opposite sexes The clinical course and severity of the respiratory distress was the same in both sexes in their series Our data is composed of a total of 406 sets of twins of which 153 sets were boys 126 sets were girls and 127 pairs were boy and girl (total 433 males and 379 females) This group has taken into account only those cases which presented a severe form of respiratory distress (Stadium II of Silverman) and cyanosis requiring the administration of oxygen

Severe respiratory distress was present in 9 93 of males and 8 7% of females This slightly higher incidence in males studied by the homogeneous test between two averages was not significant (p between 0 6 and 0 5) The number of deaths in both sexes considering the mortality during the first 10 days of life in both sexes has been similar (6 9% and 7 1) The most important anatomical findings in these cases (autopsy index 91 2%) were similar in both sexes and correspond to typical forms of atelectasis with hyaline membranes (66 6 and 68%) respectively Pulmonary immaturity and pulmonary haemorrhages were the most frequent accompanying findings

D *Macro- and microscopic post mortem examination on 700 cases* The series of 700 post mortem examinations performed on cases of children deceased during the period 0–30 days

Table 7 *Relation between sex and pulmonary maturity*

Pulmonary maturation	Total cases	Males	Females
Type III	40	18	22
Type II	55	29	26
Type I	31	16	15
Type 0	6	3	3
Total	132	66	66

of life has been classified into 3 groups corresponding to premature birth from 500 to 1 250 g premature births from 1 251 to 2 500 g and full term births with weight in excess of 2 500 g The distribution of this series is quoted in Table 3

The classification of pulmonary findings in the post mortem examinations of 439 prematures and 261 full terms death in the period 0–30 days of life is showed in Table 4

The incidence of the hyaline membrane according to weight groups showed a notable greater frequency in the 1 250 to 2 500 g weight group This incidence taking the 0–5 days death group and a comparison with different statistics from the literature is quoted in Table 5

In the 160 cases of hyaline membrane studied with a typical histological finding the associated pulmonary histological lesions apart from atelectasis and immaturity to a variable extent were as follows discreet pulmonary haemorrhages 23 7% minimum granulocytary exudation 16 2% acquired pneumonia 7 5% intrauterine pneumonia 3 1% massive pulmonary haemorrhage 2.5 aspirative syndrome 2.5% Isterious 0 6% brain lesions comprising subarachnoid haemorrhages 20 6%

With reference to the type of birth the greatest incidence of hyaline membrane occurred in prematures and in full terms delivered by cesarean section or bridge delivery with a frequency rate 3 to 6 times greater

In the 160 cases of hyaline membrane found in our series of 700 autopsies 10 6% were

Table 5 *Autopsic incidence of hyaline membrane according to weight groups compared with various previous studies*

Author	Period	Weight	Age	Autopsies	H M	H M %
Svanesen	59/60	500–2 500	1 week	304	116	38 1
Latham *et al*	37/49	1 000–2 500	1 week	257	98	38 1
Avery & Oppenheimer	44/48	1 000–2 500	30 min–6 days	70	17	24 3
Avery & Oppenheimer	54/58	1 000–2 500	30 min–6 days	145	56	38 6
Ahvenainen & Terho	54/61	600–2 500	—	174	40	23 5
Hirvensalo & Arko	61/65	600–2 500	1 week	319	68	21 3
This study	66/68	500–2 500	0–5 days	333	126	37 8
This study	66/68	500–1 250	0–5 days	116	20	17 2
This study	66/68	1 251–2 500	0–5 days	217	106	48 8
Hirvensalo & Arko	61/65	+2 500	1 week	120	15	12 5
This study	66/68	+2 500	0–5 days	190	32	16 8

in stadia I or II 2 036—overall frequency 6 4%

In prematures the frequency is much greater and also depends on the weight

The incidence was much higher in the low birth weight group and in this way in 112 cases of weight ranging between 500 and 1 250 the frequency of distress was about 90 1% , in front of a frequency of 23 9% in the group of 1 321 prematures with weight ranging between 1 251 and 2,500 g

Miller sets frequencies of 81 8% and 24 4% respectively for the groups with weight ranging between 1 000–1 500 and 2 001–2,500 g The total frequency of our series of respiratory distress (moderate and severe cases) over a total of 1 433 prematures weighing 500–2,500 g = 29 03 %

The incidence of severe forms in Silverman stadium II with cyanosis requiring permanent administration of oxygen is quoted in Table 2 Post mortem examinations were performed on all the 38 prematures weighing less than 1,250 g who died of severe respiratory distress and in the group of 44 weighing more than 1,250 g who died, post mortem examinations were performed in all except one In the group of prematures of 500–1,250 deceased during the period of 0–10 days of life, as a result of severe clinical respiratory distress and with post mortem examination the predominant alteration was pulmonary immaturity with hyaline membrane in 10 5% of the cases pulmonary haemorrhage in 10 5% and pneumonia in 26 3% In the group of 43 cases with weight ranging between 1,251 and 2 500 g death in the same period of life the main pulmonary finding was hyaline membrane with an incidence of 39 5% pulmonary haemorrhage was present in 20 4% of the cases and pneumonia only in 4 5% of them —In this latter group a 25 5% incidence of severely malformed infants was encountered

With reference to the incidence by sex of

Table 6 *Incidence of pulmonary haemorrhages*

Author	Period	Weight	Age	No of autopsies	Pulm haemorrh	Frequency in
Esterly & Oppenheimer	1954/64	0–2 500	2 weeks	668	94	14
Esterly & Oppenheimer	1954/65	+2 500	2 weeks	90	41	45 6
Ballabriga & Moragas	1966/68	500–2 500	0–10 days	383[a]	49	12 7
Ballabriga & Moragas	1966/68	+2 500	0–10 days	214[b]	38	17 7

[a] The cases of hyaline membrane and pulmonary haemorrhage have been tabulated only under the heading of hyaline membrane

[b] This group contained 21 9 of pneumonias (47/214) A large number of them offered extensive pulmonary haemorrhages but these cases have been catalogued as pneumonias

natal period varies according to different authors This depends on the strictness of the anatomical and clinical classification adopted Knecht (9) found massive intraalveolar haemorrhages in 4 2% of the live birth autopsy findings of newborns up to one month of age In 336 neonatal deaths studied by Thorburn (16) 8% pulmonary haemorrhages were found

Our data concerning pulmonary haemorrhages in prematures show an incidence of 17 7% as the cause of death similar to the data of Esterly & Oppenheimer (6) of 14

CONCLUSIONS

The incidence of respiratory distress has been higher in the males with respect to the normal ratio of male/female births the difference being significant

The highest incidence of hyaline membrane has been found in the group ranging in weight between 1 251–2 500 g (48 8 of 217 autopsies)

The incidence of intrauterine pneumonia has been higher in the group with weight ranging between 500–1 250 g and death during the first day of life

The frequency of hyaline membrane with reference to the type of delivery is higher in groups born by caesarean section or breech delivery

In the groups of deaths between 5–30 days of life pneumonia has been the main cause of death in full terms as well as in prematures

SUMMARY

The study of one minute Apgar index in 20 000 full terms babies and 933 prematures showed a frequency of the score 0–3 of 1 9% in the group of full term babies and 9 4 in the group of prematures The neonatal mortality of this material was 1 4

The incidence of respiratory distress in 31 330 full term babies classified according to the Silverman & Andersen score was 6 4%

In a group of 112 prematures with weight ranging between 500–1,250 g the incidence of respiratory distress was 90 1% and in 1 321 prematures with weight ranging between 1 251 –2 500 g was 23 9%

In relationship with the incidence of respiratory distress in twins the study of 406 sets of twins shows that it has not been possible to observe a predominance of severe forms in either sex likewise the number of deaths in both sexes has been similar considering the mortality during the first 10 days of life

Histological examination in 700 autopsies (deaths in the period 0–30 days of life) showed that in a group of 333 prematures with deaths in the period 0–5 days of life the incidence of hyaline membrane was 37 4% and in a group of 190 full terms autopsied in the same period of life was 17 this difference being significant

The frequency of hyaline membrane with reference to the type of delivery shows a high incidence in groups born by caesarean section or breech delivery The incidence of massive pulmonary haemorrhages was 12 7 % among 383 autopsies of prematures and 17 7 % among 214 full term babies autopsied during the period 0–10 days of life

Incidence of listeriosis as main cause of death in 700 post mortem examinations performed during the period 0–30 days of life was 1 71%

REFERENCES

1 Ahvenainen E. K. & Terho A Mortality of low birth weight in infants in a General Hospital *Ann Paediat Fenniae 14* 11 1968
2 Apgar V The newborn (Apgar) scoring system Reflection and advice *Pediat Clin N Amer 13* 645 1966
3 Apgar V & James L. S Further observations on the newborn scoring system *Amer J Dis Child 104* 419 1962
4 Avery M E. & Oppenheimer E H Recent increase in mortality from hyaline membrane disease *J Pediat 57* 553 1960
5 Drage J S Kennedy C & Schwartz B K. The apgar score as an index of neonatal mortality *Obstet Gynec 24* 222 1964
6 Esterly J R. & Oppenheimer E H Massive pulmonary haemorrhage in the newborn *J Pathologic considerations J Pediat 69* 3 1966

born by ceasarean section and 17 5% by *breech delivery meanwhile the usual percent* age of ceasarean section and breech delivery in the total deliveries of our Maternity Hospital arounded 2 9* and 2 6*₀ *respectively*

The incidence of pulmonary haemorrhages in the post mortem examinations in our series have been compared with the series of Esterly & Oppenheimer (6) and results have been quoted in Table 6

The relation between sex and pulmonary ma turity has been examinated in 132 lungs from prematures ranging weight between 500 and 1,250 g and the results are quoted in Table 7

The sex ratio in all the cases of respiratory distress observed has been 1 male/0 717 fe male and in pulmonary immaturity the ratio has been 1 male/1 female

DISCUSSION

In relationship to the data obtained with the one minute Apgar score (2 3) in our series and in comparison with various previous stud ies, *it is seen that there is a greater number of* asphyctic children at the time of birth in the two sets of American statistics than in the European ones, but a greater number of them may be recovered with a lower mortality rate This may be because in the European series the administration of analgesics and sedatives is perhaps less and therefore the cases with low scores in our series correspond to very severe forms with a high mortality rate

On the other hand *in the series of prema* ture infants with scores between 7 and 10 the mortality rate is notably higher if compared with the full terms offering the same score and this must be considered from the point of view the postnatal mortality of prematures during the first month of life is influenced by other factors The prognosis with reference to mor tality established by the Apgar index is better for full term infants than premature ones

Concerning pulmonary findings as cause of death the lung has been classified according to the most important and extensive feature but very frequently this primary cause is mixed and there are several factors which can contri bute as important associated causes The in cidence of the hyaline membrane according *to weight groups showed a notable greater fre* quency in the 1,250 to 2,500 g group

The association between hyaline membran and intrauterine pneumonia has been an excep tion in our series, on the other hand the associ ation between fragmented hyaline membranes in period of reabsorption and acquired pneu monia have been frequent in the group of deaths occurring between 48 hours and 10 days of life

Pulmonary immaturity has been frequently associated with atelectasis and interstitial em physema, likewise there is a frequent associa tion between hyaline membrane atelectasis, interstitial emphysema and immaturity of vari able degree

The frequency of hyaline membrane in the series of prematures dying in the period 0-5 days of life (37 53*₀) compared to the fre quency in full terms dead in the same period (16 8%) was significantly (*p*<0 001) higher

The difference in frequency of pulmonary haemorrhages and pneumonias in the period 0–5 days of life between prematures and full terms was not significant (*p*>0 05) Neither exists a significant difference (*p*>0 05) be tween the percentage of pneumonia in the pe riod 5–30 days of life in prematures (58 49 •) and full terms (56 33%)

Pulmonary immaturity in its two advanced grades *types II and III was frequently* asso ciated with intrauterine pneumonia while the hyaline membrane is more commonly associ ated with the groups grade 0 and I of pul monary maturity

The incidence of respiratory distress has been higher in the males with respect to the normal ratio of male/female birth and con firming Landing s (10) findings that pulmonary immaturity shows a slight predominance in the *female sex*

The incidence of pulmonary haemorrhages in the post mortem materials from the post

natal period varies according to different authors. This depends on the strictness of the anatomical and clinical classification adopted. Knecht (9) found massive intraalveolar haemorrhages in 4.2% of the live birth autopsy findings of newborns up to one month of age. In 336 neonatal deaths studied by Thorburn (16) 8% pulmonary haemorrhages were found.

Our data concerning pulmonary haemorrhages in prematures show an incidence of 17.7% as the cause of death, similar to the data of Esterly & Oppenheimer (6) of 14%.

CONCLUSIONS

The incidence of respiratory distress has been higher in the males with respect to the normal ratio of male/female births, the difference being significant.

The highest incidence of hyaline membrane has been found in the group ranging in weight between 1 251–2 500 g (48.8% of 217 autopsies).

The incidence of intrauterine pneumonia has been higher in the group with weight ranging between 500–1 250 g and death during the first day of life.

The frequency of hyaline membrane with reference to the type of delivery is higher in groups born by caesarean section or breech delivery.

In the groups of deaths between 5–30 days of life pneumonia has been the main cause of death in full terms as well as in prematures.

SUMMARY

The study of one minute Apgar index in 20 000 full terms babies and 933 prematures showed a frequency of the score 0–3 of 1.9 in the group of full term babies and 9.4 in the group of prematures. The neonatal mortality of this material was 1.4.

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Histological examination in 700 autopsies (deaths in the period 0–30 days of life) showed that in a group of 333 prematures with deaths in the period 0–5 days of life, the incidence of hyaline membrane was 37.4% and in a group of 190 full terms autopsied in the same period of life was 17 ..., this difference being significant.

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Incidence of kernicterus as main cause of death in 700 post mortem examinations performed during the period 0–30 days of life was 1.71.

REFERENCES

1. Ahvenainen, E. K. & Turbo, A. Mortality of low birth weight in infants in a General Hospital. Ann Paediat Fenniae 14: 11, 1968.
2. Apgar, V. The newborn (Apgar) scoring system. Reflection and advice. Pediat Clin N Amer 13: 645, 1966.
3. Apgar, V. & James, L. S. Further observations on the newborn scoring system. Amer J Dis Child 104: 419, 1962.
4. Avery, M. E. & Oppenheimer, E. H. Recent increase in mortality from hyaline membrane disease. J Pediat 57: 553, 1960.
5. Drage, J. S., Kennedy, C. & Schwartz, B. K. The Apgar score as an index of neonatal mortality. Obstet Gynec 24: 222, 1964.
6. Esterly, J. R. & Oppenheimer, E. H. Massive pulmonary haemorrhage in the newborn. I. Pathologic considerations. J Pediat 69: 3, 1966.

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Pulmonary immaturity in its two advanced grades types II and III was frequently associated with intrauterine pneumonia while the hyaline membrane is more commonly associated with the groups grade 0 and I of pulmonary maturity

The incidence of respiratory distress has been higher in the males with respect to the normal ratio of male/female birth and confirming Landing s (10) findings that pulmonary immaturity shows a slight predominance in the female sex

The incidence of pulmonary haemorrhages in the post mortem materials from the post

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THE FATTY ACID COMPOSITION OF DEPOT FAT IN CHILDHOOD

1 *The effect of age sex and site in superficial fat*

JOHN A BIRKBECK

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The fatty acid composition of the storage triglycerides of human depot fat has been reported by several workers particularly since the introduction of the precise analytical technique of gas liquid chromatography (19) Some authors (e g 31) have regarded human depot fat as relatively uniform in fatty acid composition although there is much evidence to the contrary

In the adult, there is little evidence that in health the pattern changes with age However several studies of the newborn period have demonstrated major differences from the adult pattern Such differences are commonly ascribed to the effect of different dietary lipid composition although they are demonstrable prior to feeding at birth and vary between full term and premature infants

Studies of subjects from birth to maturity are scanty The present report deals with an investigation into the influence of age sex and sampling site on the fatty acid pattern of subcutaneous depot fat in children

METHODS

The study population consisted of individuals between birth and maturity admitted to a Children's Hospital for minor elective surgical procedures Most of these were hernia repairs minor orthopedic manipulations and plastic repairs All subjects were judged by examination to be otherwise healthy and free of any evidence or family history of metabolic disorder Although long term dietary habits do influence depot fat composition it was not deemed practicable to undertake retrospective dietary assessments on the subjects So far as could be determined all were eating typical" mixed North American diets

It was felt that the occasional individual with minor peculiarities of diet habit would affect the overall results very little due to the large sample size Most of the subjects were of Caucasian origin the few Orientals were on a North American type of diet There were 48 satisfactory analyses of tissue from the anterior abdominal wall (38 male and 10 female) and 35 samples from limb sites (8 male and 27 female)

Fat samples typically 0 1 to 0 5 g in weight from accessible sites at surgery were removed cleanly by the surgeon at the start of the operation The sample was immediately placed in a tube with sterile saline containing hydroquinone as an antioxidant and frozen as soon as practicable A preliminary study failed to reveal any effect of the storage procedure the hydroquinone or the duration of storage on the analytical results

A small (30-50 mg) sample of the tissue was minced The lipids were hydrolysed and the fatty acids converted to their methyl esters by the method of Metcalfe *et al* (29) as modified by Endres (6) The hydrolysis and esterification were always carried out immediately prior to chromatography as it was found that storing the esters at room temperature gave unreliable results In this way close replication was achieved

The ester mixture was analysed on a F & M model 810 dual column gas chromatograph using hydrogen flame ionization detectors The columns were 10 % EGSS-X on a support of Gas-Chrom P (100–120 mesh) the aluminum column being 8 feet in length and / inch o d The carrier gas was helium Temperature programming between 150 and 200 C was employed

The peak areas were quantitated with a Disk integrator and the results expressed on the basis of relative molar response as a percentage of the total (36) That is the quantitation was corrected for small differences in detection response to acids of differing

7 Hirvensalo, M & Arko H Studies in perinatal mortality in a large Maternity Hospital *Ann Paediat Fenniae 13* 46 1967
8 Ikonen R S The Apgar scoring of newborn infants and its relation to neonatal mortality *Ann Paediat Fenniae 13* 111 1967
9 Knecht U Die intraalveolare Massenblutung des Neugeborenen *Helvet Paediat Acta 21* 268 1966
10 Landing B H Pulmonary lesions of newborn infants A statistical study *Pediatrics 19* 217 1957
11 Latham E F Nesbitt R E L & Anderson G W A clinical pathological study of newborn lung with hyaline like membranes *Bull Hopkins Hosp 96* 173 1955
12 Miller H C Respiratory distress syndrome of newborn infants 1 Diagnosis and incidence *J Pediat 61* 2 1962
13 Miller H C & Futrakul P Birth weight gestational age and sex as determining factors in the incidence of respiratory distress syndrome of prematurely born infants *J Pediat 72* 628 1968
14 Silverman W A & Andersen D H A controlled clinical trial of effects of water mist on obstructive respiratory signs death rate and necropsy findings among premature infants *Pediatrics 17* 1 1956
15 Srivanesen S Neonatal pulmonary pathology Singapore *J Pediat 59* 600 1961
16 Thorburn M J Neonatal death and massive pulmonary haemorrhage in Jamaica *Arch Dis Childh 38* 589 1963

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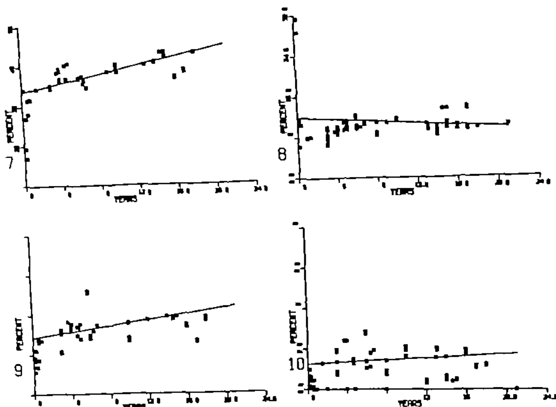


Fig. 1. Relationship of fatty acid composition with age. *Ordinate:* fatty acid as percent of total; abscissa: age in years. Graph 1 is 12:0; 2, 14:0; 3, 16:0; 4, 16:1; 5, 17:0; 6, 18:0; 7, 18:1; 8, 18:2; 9, 18:3; 10, 20:4.

decanoic acid (17 : 0) was used as an internal standard but with the routine use of standard mixture (the NIH standards plus Standard No. 16) for calibration the instrument was found to be sufficiently stable that retention time alone was satisfactory for identification. For a more detailed analysis of the minor and trace components of human depot fat, the reader is referred to the meticulous studies of Kingsbury and colleagues (21, 22).

RESULTS

The analyses of 83 samples of subcutaneous fat were reviewed. Since it was possible that age, sex, and site of sampling might be relevant variables, the data was subjected to computer analysis.

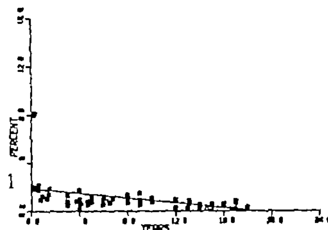
The reproducibility of the method was very satisfactory. Every sample was analysed at least twice, and most often three times. Replication was within the limits found for the NIH standards, in fact within 1% for major peaks and 2.5% for others.

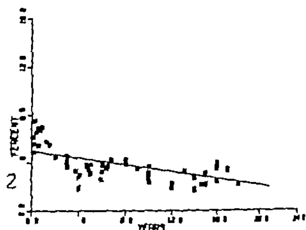
The fatty acids identified were 10:0, 12:0, 14:0, 16:0, 16:1, 17:0, 18:0, 18:1, 18:2, 18:3 and 20:4. No identification of the other trace peaks was attempted. Although identified, 10:0 represented less than 0.1% of the total in 83% of the samples, and never more than 0.3%. Accordingly, values for this acid were not analysed further.

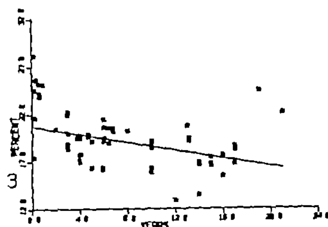
The unknown peak, originally thought to be 20:0, represented from a trace to as much as 1.7% of the total; accordingly has been taken into consideration in the calculations.

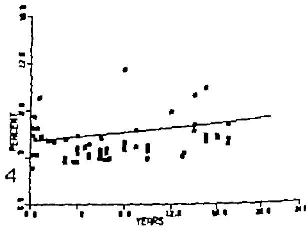
Representative mean results for individuals of each sex at different ages, in anterior abdominal wall fat, are given in Table I. General trends are discernible in these selected figures, but analysis of this amount of data proved to be highly complex.

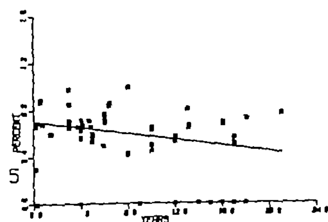
A two-way computer analysis of covariance with one observation (i.e. the mean) per cell

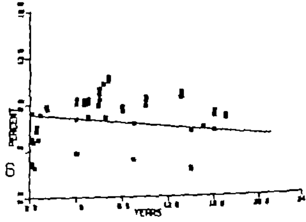












structure by comparison with standards of known composition Although other esters such as those of cholesterol or phospholipids might contribute to the fatty acids in the mixture it was felt that such an effect would be negligible since over 99 of adipose tissue lipid has been shown to be triglyceride (20)

Utilizing NIH standards A B C and D replicate samples agreed with the stated composition to within 3 for major peaks (greater than 10 of the total) and within 3 5 for minor peaks (less than 10 of the total) (16)

The analytic system could not readily distinguish between the peaks of the 18 3 and 20 0 esters an accordingly the level for the former included trac of the latter A minor peak originally identified 20 0 was found to be distinguishable from it an has not been positively identified

Several trace peaks (defined as less than 0 1 the total) were consistently seen They were not cluded in the calculations as collectively they woul not influence the results significantly Initially hept

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The number before the colon refers to the chai length that after to the degree of unsaturation

Table 3 *Fatty acid composition of milk triglycerides*

Composition expressed as percentage

Fatty acid	4 0	6 0	8 0	10 0	12 0	14 0	16 0	16 1	18 0	18 1	18 2	20 0
Bovine milk	10 2	2 5	1 3	1 5	3 3	8 6	21 1	2 8	9 9	31 4	4 9	0 7
Human milk	—	—	—	2 1	7 7	9 0	23 6	5 4	6 5	33 2	7 1	0 9

Bovine data from Hilditch & Paul 1940 Human data from Hilditch & Meara 1944 See also Hilditch & Williams (12)

Only a few papers have examined trends throughout childhood (1 8 23 34) and these in general have shown that the proportion of 16 0 and sometimes of 16 1 and 18 0 is much higher in depot fat from newborns than in older children while the proportion of 18 2 is lower Takazawa (34) reports that the ratio of unsaturated to saturated fatty acid content increases with increasing age When considering these findings it must be remembered that since the figures are always expressed as percentages a change in the level of one component particularly a major one inevitably changes the values for all the rest this adds to the complexities of analysing this type of data

Fujimoto (8) showed a continuing but gradual change from infancy until about 7 or 8 years of age by which time values similar to the adult were reached

The present results confirm in general the reported values but extend them Levels of 12 0 and 18 2 are strikingly higher in infancy levels of 14 0 and 16 0 are higher and levels of 18 1 are much lower than in older children while levels of 18 3 are somewhat lower Most of the trend toward adult levels appears to have occurred by about 5 years of age Levels of 20 4 seem highly variable

An age trend in adults was reported by Insull & Bartsch (18) In a large series ranging from adolescence to 79 years the proportion of 18 1 was said to increase with age while that of 12 0 14 0 and 18 0 tended to decrease Such observations may influence the interpretation of conclusions reached in adult studies on small populations

Sex The majority of reports in the literature did not consider a possible effect of sex on composition McLaren *et al* (26) did not observe any sex difference in a small and heterogenous population Pietropaolo et al (31) in a study of adults concluded that "depot fat in man presents an almost uniform make up Although they make no specific comment their data shows no sex effect

Kirot & Bronte Stewart (24) studying adults of varying racial origin reported that males had significantly higher content of 16 0 and 18 0 in their depot fat while females had more 16 1 and 18 1 However these sex differences varied in the various racial groups studied Since at least 16 0 and 18 1 are major components of depot fat these observations would suggest that female fat is more unsaturated that is "softer than male since unsaturation for a given chain length reduces melting point

Insull & Bartsch (18) found in their adult autopsy specimens that males had slightly higher 18 0 content than females ($p < 0.05$) while females had more 18 1 (n.s.) In view of the common failure to take factors such as age and in some cases sampling site into consideration drawing definite conclusions on the basis of published figures is hazardous and the possibility of sex differences in adults or in children remains

Site Many authors had sampled from only one site or did not report the sites from which the samples were taken We have not investigated in this present study possible differences in composition between superficial and deep depot fat

Innachi et al (17) reported that distal limb sites showed significant compositional differences from abdominal wall sites the latter having more 14 0 16 0 and 18 0 and less 14 1 and

Table 1 *Analyses of superficial fat from typical subjects at different ages (mean values)*

Acid	12 0	14 0	16 0	16 1	17 0	18 0	18 1	18 2	18 3	20 4
Males										
3 months	8 1	7 5	21 6	4 3	0 0	2 7	23 0	28 7	1 4	0 6
1½ years	1 9	5 5	21 0	5 4	0 6	7 7	40 4	10 2	3 3	1 3
3 years	1 3	4 6	22 3	4 0	0 7	10 5	40 7	7 8	2 2	1 4
5 years	0 8	3 0	16 4	4 3	0 6	8 1	51 5	11 0	3 6	2 6
15 years	0 6	3 2	16 6	5 9	0 0	7 3	45 4	9 7	5 9	3 9
Females										
2 months	7 8	6 2	17 4	3 1	0 3	3 0	25 3	31 3	1 1	0 7
17 months	1 4	3 3	17 6	4 6	0 6	7 9	43 6	13 1	2 8	0 7
3 years	0 8	4 1	18 9	5 6	0 7	8 0	42 4	8 4	4 2	3 0
5 years	0 7	3 5	17 8	9 2	0 7	3 7	48 2	9 8	3 4	0 0
14 years	0 5	2 7	16 7	10 8	0 0	2 5	51 1	9 1	3 2	0 7

was undertaken on the IBM 360 computer taking into account age sex and subcutaneous site (i e abdominal wall or limb) Unfortunately as the data were of course collected in a random fashion cell sizes were quite unequal, which tended to reduce the potency of the mathematical technique Since making the cell size more equal, either by reducing the cells to the lowest common value or selectively accumulating further data to enlarge the smaller cells would also disturb the validity of the method no changes have been made It is possible that minor degrees of correlation may have been missed as a consequence

Effect of age The levels of 12 0 14 0 16 0 and 18 2 are much higher in infancy than in adult life and diminish in most instances rapidly at first and then more slowly in mid childhood Conversely the level of 18 1 is much lower in infancy and rises thereafter The regression coefficients for these acids by age (sex and site being kept constant) are shown in Table 2 Although the curve of mean values would frequently be better represented by a curve the complexity of analysis required was not felt to be justified and so straight lines have been fitted

Table 2 *Regression data for fatty acid composition*

Fatty acid	Slope s (per year)	Intercept i ( )
12 0	−0 10039	1 885
14 0	−0 14164	4 966
16 0	−0 21138	20 741
16 1	0 09031	5 460
17 0	−0 01301	0 704
18 0	−0 09281	7 171
18 1	0 50834	39 740
18 2	−0 11522	12 052
18 3	0 07038	2 875
20 4	0 02267	1 274

y = fatty acid percentage x = age in years $y = sx + i$

Effect of sex No effect of sex on composition could be detected when the other variables were controlled

Effect of subcutaneous site No difference in composition between samples from anterior abdominal wall and from limb sites was demonstrated This is at variance with some evidence in the literature and may reflect the fact that most limb samples were from relatively proximal limb sites

DISCUSSION

Age The topic of human depot fat composition has received considerable attention in recent years and although the majority of reports deal with samples from adult subjects (10 17 18 22 24 31) several have dealt with samples from children particularly in the newborn period In two reports the age of the subjects was not specified (21 27) but was assumed to be adult Dramatic differences have been shown between the composition of depot fat in the newborn as compared to either normal adult controls (1 3 26) or the infant

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Insinchi *et al* (17) reported that distal limb sites showed significant compositional differences from abdominal wall sites the latter having more 14 0 16 0 and 18 0 and less 14 1 and

16 1 than the former They speculate that the differences may be related to physical properties required by the site more peripheral and thus environmentally cooler sites bearing fat of lower melting point (more unsaturated) than central

Unfortunately the data are drawn from a very small sample (1 female and 5 male adults of widely disparant ages) They conclude that since all the fatty acids concerned can be synthesised de novo by the adipose tissue, the differences may be related to different synthetic preferences between the tissues

Other authors (10 26 27) report only that leg depot fat has less 18 0 and more 16 1 than abdominal wall fat, the composition being other wise similar

Other Factors That diet can influence depot fat composition is well established (13 14) At least in the adult the effect of a major change in dietary fat composition is reflected only very slowly in the depot fat Hirsch (13) estimates the half time of exchange between dietary (and therefore plasma (7)) and depot triglyceride fatty acids at 350–750 days However in periods of rapid weight gain such as early infancy or during realimentation after severe malnutrition much more rapid changes can occur Feeding corn oil to a nursing mother produced immediate changes in maternal milk composition and the infant s depot fat reflected the change within 10 days (14) Sweeney (33) has also observed the same trend in small infants during rapid weight gain

McLaren *et al* (16) however on the basis of their studies in different ethnic groups in Africa doubt that diet is a primary factor in depot fat composition The subjects studied by Pietropaolo *et al* (31) in Italy had higher levels of 18 1 than usually reported and this was felt to be a dietary effect

After the period of infancy (say the first year of life) most children in North America consume foods more or less identical with those of the adults of the family

Accordingly if we are to seek dietary factors to explain the gradation of composition through childhood it is more likely a quantitative rather than a qualitative effect The most obvious possibility is, of course, the consumption of bovine milk Children consume much more milk than adults many of whom consume very small amounts only

The composition of typical bovine and human milk fats are shown in Table 3 It will be observed that relative to adult depot fat composition bovine milk contains more 12 0 14 0 and 16 0 but less 18 1 and 18 2 Compared with infant depot fat bovine milk contains rather less 12 0 (although the difference could be accounted for by lengthening the chain of some of the shorter fatty acids prominent in milk) about the same amount of 14 0 and 16 0 much more 18 0 and about the same amount of 18 1 Thus far the relationship appears promising but milk contains much less 18 2 than depot fat of infants, which is particularly rich in this essential fatty acid Of course if chain length alteration is invoked to explain such differences, the significance of the similarity between diet and depot fat is lost

Little is known about hormonal influences Sex differences in fatty acid metabolism have been reported (4 9) There is no evidence of selective mobilization of any specific fatty acids in response to lipolytic agents (30)

Environmental temperature might be a factor as suggested by Henriques & Hansen in the hog (11) and Imiachi *et al* (17) in man Several studies from Africa have claimed that environmental temperature changes are responsible for differences in depot fat composition (25 26)

In view of the dearth of unequivocal data regarding the effect of factors other than diet on depot fatty acid composition speculation regarding the significance if any of such composition remains speculative Since there seems little doubt that a major proportion of the fatty acids in the mammalian system are synthesised from dietary *carbohydrate* sources (26) just why dietary fatty acid patterns should apparently influence composition so much is difficult to explain Foods rich in *carbohydrate* are said to

favour the deposition of saturated fatty acids especially stearic (18 0) (20 31 35) Indeed in the human adult although adipose tissue is a major site of lipogenesis from glucose at times of glucose excess at other times the liver appears predominant (2) Further in adipose tissue glyceride glycerol is probably the major product rather than fatty acid (2 15) Whether this holds true in the metabolically more active child is not established

Studies in the last decade have suggested that depot fat is not a torpid storehouse for excess calories but an active metabolic system providing rapid fluxes of energy either as fatty acids or glucose inwards in times of alimentation and outwards as fatty acids and glycerol in times of fasting or great metabolic need If this is so it is surprising that dietary changes should take such a very long time to affect composition This might suggest compartmentalisation of the depots with a large inert mass and small active one There is no evidence of such differentiation in man

Recent studies have shown that there are fatty acid families possibly important in the mammalian economy which were previously unrecognised (32) It is clear that future studies must concentrate on the relationship of the cellular environment to the composition of the fat being stored rather than just the static composition thereof

SUMMARY

In childhood age has a major influence on the fatty acid composition of superficial adipose depot triglycerides Although the effect is most marked in the newborn period it continued at least to mid-childhood After correcting for this variable no difference between male and female was apparent and no significant difference was observed between anterior abdominal wall and limb fat It seems unlikely that diet alone can explain these differences Our understanding of other possible factors in the determination of fatty acid distribution in storage fat is meagre

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REFERENCES

1 Baker G L Human adipose tissue composition and age *Am J Clin Nutr* 22 829 1969
2 Bjorntorp P Schersten T Gottfries A Effects of glucose infusions on adipose tissue lipogenesis in man *Acta Med Scand* 183 656 1968
3 Bondrup K Koster N Quaade F & Rosreen H Gas chromatography analysis of human subcutaneous fat *Scand J Clin Lab Invest* 18 588 1966
4 Christiansen K Gan M V & Holman R T Sex differences in the metabolism of fatty acids in vitro *Biochim Biophys Acta* 187 19 1969
5 Dawkins M J R & Stevens J T Fatty acid compositions of triglycerides from adipose tissue *Nature* 209 1145 1966
6 Endres J G An apparatus for the rapid preparation of fatty acid esters from lipids for gas chromatography analysis *J Amer Oil Chem Soc* 43 600 1966
7 Fleischmann A I Rayton T & Berenbaum K L Variations in the composition of free fatty acids with dietary change under nocaloric conditions *Am J Clin Nutr* 15 299 1964
8 Fujimoto E Studies on the fatty acid composition of body fat with particular reference to body weight and age *Acta Paediat Jap* 8 36 1966
9 Gilmour K F & McKerns K W Insulin and estrogen regulation of lipid synthesis in adipose tissue *Biochim Biophys Acta* 116 220 1966
10 *Hilditch A O A The fatty acid composition* of human adipose tissue *Clin Sci* 25 433 1963
11 Henriques V & Hansen C Comparative studies on the chemical composition of animal fat *Skand Arch Physiol* 11 151 1901
12 Hilditch T P & Williams P N *The chemical constitution of natural fats* Chapman and Hall London 4th edn 1964 p 118
13 Hirsch J Fatty acid patterns in human adipose tissue *In Handbook of Physiology* Section 5 Adipose Tissue Amer Physiol Soc 1956 p 181
14 Hirsch J Farquhar J W Ahrens E H Peterson M L Stoffel W Studies of adipose tissue in man *Am J Clin Nutr* 8 499 1960
15 Hirsch J & Goldrick J B Serial studies on the metabolism of human adipose tissue I *J Clin Invest* 43 1776 1964

16 Hornung E C Ahrens E H Lipsky S R Mattson F H Mead J F Turner D A & Goldwater W H Quantitative analysis of fatty acids by gas liquid chromatography *J Lipid Res* 5 20 1964
17 Imaichi K Fukuda J Oyama K & Mukawa, A Effect of sample site on fatty acid composition of adipose tissue *J Biochem (Tokyo)* 58 463 1965
18 Insull W & Bartsch G E Fatty acid composition of human adipose tissue related to age se and race *Am J Clin Nutr* 20 13 1967
19 James A T & Martin A J P Gas liquid chromatography the separation and identification of the methyl esters of unsaturated and saturated fatty acids from formic acid to n octadecanoic acid *Biochem J* 63 44 1956
20 Jeanrenaud B Lipid components of adipose tissue In *Handbook of Physiology* Section 5 Adipose Tissue Am Physiol Soc 1956 p 169
21 Kingsbury K J Morgan D M & Heyes T D The analysis of the fatty acids of normal human depot fat by gas liquid chromatography *Biochem J* 90 140 1964
22 Kingsbury K J Paul S Crossley A & Morgan D M The fatty acid composition of human depot fat *Biochem J* 78 541 1961
23 Kondo T Studies on the fatty acid composition of the bone marrow fat in childhood *Acta Paediat Jap* 9 86 1967
24 Krut L H & Bronte Stewart B The fatty acids of human depot fat *J Lipid Res* 5 343 1964
25 MacDonald I Ambient temperature and depot fat iodine number of children *Nature* 192 353 1961
26 McLaren D S Ajam Z A & Awdeh Z Composition of human adipose tissue with special reference to site and age differences *Am J Clin Nutr* 17 171 1965
27 McLaren D S & Read W W C Fatty acid composition of adipose tissue *Clin Sci* 23 247 1962
28 Martinsson A On the composition of human adipose tissue *Acta Med Scand* 182 795 1967
29 Metcalfe L D Schmitz A A & Pelka J R Rapid preparations of fatty acid esters from lipids for gas chromatography analysis *Analyt Chem* 38 515 1966
30 Norcia L N & Evans J D Relation of plasma free fatty acid composition to the origin of free fatty acids from depot fat triglycerides *Metabolism* 13 12 1964
31 Pietropaolo C Pisano, L & Cali G Fatty acid composition of human adipose tissue in different sites *Clin Chim Acta* 10 485 1964
32 Schlenk H & Sand D M A new group of essential fatty acids and their comparison with other polyenoic fatty acids *Biochim Biophys Acta* 144 305 1967
33 Sweeney M J Etteldorf J N Throop L J Timma D L & Wrenn E L Diet and fatty acid distribution in subcutaneous fat and in the triglycerides cholesterol fraction of serum of young infants *J Clin Invest* 42 1 1963
34 Takizawa Y Studies on the fatty acid composition of subcutaneous fat in childhood *Acta Paediat Jap* 9 87 1967
35 Vague J The degree of masculine differentiation of obesities a factor determining predisposition to diabetes atherosclerosis gout and uric calculous disease *Am J Clin Nutr* 4 1 1956
36 Wilson R H Doty V E Rencz K H & Schram A C Gas chromatographic analysis of high molecular weight fatty acid methyl esters with the technique of relative molar response *J Lab Clin Med* 67 87 1966

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MAXIMUM TUBULAR REABSORPTION OF WATER

A Preliminary Study of Renal Concentrating Capacity in Children with Cronic Pyelonephritis

H C SOMMERSCHILD and G GROTTE with technical assistance by KATARINA HALLIN

From the Department of Pediatric Surgery University Hospital Uppsala Sweden

The concentrating capacity is assumed to be the first of the kidney functions affected in recurrent urinary infections. This capacity is usually expressed as maximum urinary specific gravity during 14–16 hours d hydration. Winberg (15) has shown that normal osmolality should exceed 800 mOsm/l and that children reach this capacity during the third year of life. However these concentration tests based upon urinary specific gravity measurements have a significant rate of error.

Another method of determining renal concentration capacity is by induced osmotic diuresis in hydropenic individuals. Osmotic diuresis is effected by intravenous infusion of a substance such as mannitol which is filtrated in the glomeruli but is not reabsorbed in the nephrons. In this way the ability of the kidney to build up interstitial medullary hyperosmolality may be tested. This may be expressed as osmotic clearance (Cosm) using the total amount of cleared osmotically active substance in the classical definition of renal clearance. The more effective this ability is the greater the amount of water reabsorbed from the collecting tubules will be. The amount of water reabsorbed in excess of the amount corresponding to the reabsorbed osmotically active substances is termed "reabsorbed free water" ($T^{c}_{H_2O}$). In maximum the magnitude of Cosm and diuresis is the same and $T^{c}_{H_2O}$ 0. Each increase in diuresis is accompanied by an equal increase in Cosm thereby creating an isosthenuric curve or base line (Fig 1) (vide infra). These parameters may be expressed as follows.

The purpose of this investigation was to determine the possibility of detecting impaired renal concentration capacity at an earlier stage by use of mannitol induced osmotic diuresis than is possible by the conventional specific gravity measurements.

MATERIAL AND METHODS

Eleven patients from 2 13 years of age were investigated. Ten of these patients had recurrent urinary infections with acute relapses during the 1 5 year observation period. Four of these ten showed normal renal function as far as could be assessed three had depressed concentrating capacity of varying severity and three had more uria and marked parenchymal changes in IVP (Table 1). No urinary system obstruction and no residual urine were observed in these patients. They were all referred to the pediatric surgical clinic for evaluation of surgical intervention. One patient was treated for hypospadia.

Method At 6 00 p m on the day before the investigation the patient was given vasopressin (Pitressin tannate in oil Parke Davis) 0 5 I U /6 kg body weight i m. Fluids were then withheld during the following 14 hours. In the morning an indwelling bladder catheter was connected to a sample bottle. At 8 00 a m the urine was collected for 30 min. During the next 2 hours 10 % mannitol was intravenously infused at a constant rate of 3 mosm/kg/hour urine specimens were collected at 15 min intervals and a blood sample taken at the same time.

Analyses The total osmolality in the urine and serum was estimated by the freezing point technic using a Knauer osmometer. Sodium and potassium in the blood and urine were analysed by flame photometry while chloride and creatinine were determined according to the Technicon autoanalyzer methodology.

16 Hornung E C Ahrens E H Lipsky S R Mattson F H Mead J F Turner D A & Goldwater W H Quantitative analysis of fatty acids by gas liquid chromatography *J Lipid Res* 5 20 1964
17 Imaichi K Fukuda J Oyama K & Mukawa A Effect of sample site on fatty acid composition of adipose tissue *J Biochem (Tokyo) 58* 463 1965
18 Insull W & Bartsch G E Fatty acid composition of human adipose tissue related to age sex and race *Am J Clin Nutr 20* 13 1967
19 James A T & Martin A J P Gas liquid chromatography the separation and identification of the methyl esters of unsaturated and saturated fatty acids from formic acid to *n* octadecanoic acid *Biochem J 63 44 1956*
20 Jeanrenaud B Lipid components of adipose tissue In *Handbook of Physiology* Section 5 Adipose Tissue Am Physiol Soc *1956* p 169
21 Kingsbury K J Morgan D M & Heyes T D The analysis of the fatty acids of normal human depot fat by gas liquid chromatography *Biochem J 90* 140 1964
22 Kingsbury K J Paul S Crossley A & Morgan D M The fatty acid composition of human depot fat *Biochem J 7?* 541 1961
23 Kondo T Studies on the fatty acid composition of the bone marrow fat in childhood *Acta Paediat Jap* 9 86 1967
24 Krut L H & Bronte Stewart B The fatty acids of human depot fat *J Lipid Res* 5 343 1964
25 MacDonald I Ambient temperature and depot fat iodine number of children *Nature 192* 353 1961
26 McLaren D S Ajans Z A & Awdeh Z Composition of human adipose tissue with special reference to site and age differences *Am J Clin Nutr 17* 171 1965
27 McLaren D S & Read W W C Fatty acid composition of adipose tissue *Clin Sci 23* 247 1962
28 Martinsson A On the composition of human adipose tissue *Acta Med Scand 182* 795 1967
29 Metcalfe L D Schmitz A A & Pelka J R Rapid preparations of fatty acid esters from lipids for gas chromatography analysis *Analyt Chem 38 515 1966*
30 Norcia L N & Evans J D Relation of plasma free fatty acid composition to the origin of free fatty acids from depot fat triglycerides *Metabolism 13* 12 1964
31 Pietropaolo C Pisano L & Cali G Fatty acid composition of human adipose tissue in different sites *Clin Chim Acta* 10 485 1964
32 Schlenk H & Sand D M A new group of essential fatty acids and their comparison with other polyenoic fatty acids *Biochim Biophys Acta 144 305 1967*
33 Sweeney M J Etteldorf J N Throop L J Timma D L & Wrenn E L Diet and fatty acid distribution in subcutaneous fat and in the triglycerides cholesterol fraction of serum of young infants *J Clin Invest 42* 1 1963
34 Takizawa Y Studies on the fatty acid composition of subcutaneous fat in childhood *Acta Paediat Jap 9 87 1967*
35 Vague J The degree of masculine differentiation of obesities a factor determining predisposition to diabetes atherosclerosis gout and uric calculous disease *Am J Clin Nutr* 4 1 1956
36 Wilson R H Doty V E Rencz K H & Schram A C Gas chromatographic analysis of high molecular weight fatty acid methyl esters with the technique of relative molar response *J Lab Clin Med 67 87* 1966

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Table 2 *Highest and lowest value of different parameters during the investigation*

No	Age year	Na/s mEq/l Max Min	Osm/s mOsm/l Min–Max	Osm/urine mosm/l Max–Min	Diuresis ml/m/1 73 m Min–Max	C_{osm}^{max} ml/m/1 73 m
1	3	138–125	270–350	720–420	0 6–6 6	13 6
2	5	136–127	280–340	810–540	1 0–5 0	9 0
3	5	137–122	270–295	980–515	0 8–7 6	13 5
4	7	145–130	290–308	970–550	1 0–5 6	10 6
5	5	147–124	307–331	1010–538	0 5–5 0	8 5
6	2	140–129	270–300	660–422	0 7–5 7	8 5
7	3	144–124	264–333	750–270	0 7–4 4	5 2
8	4	144–120	290–350	540–370	0 7–5 1	7 1
9	2	140 127	280–333	400–315	0 9–6 0	6 0
10	5	148–137	290–350	320–320	0 3–7 4	7 0
11	13	140–125	260–345	320–370	1 2–6 3	6 3

Minimum diuresis refers to the 30 min s period before starting mannitol infusion
Maximum urinary concentration also refers to this period

ment but should always remain above a mini mum level In healthy individuals a stable level of $T^c_{H_2O}$ is found when the diuresis is 4–5 ml/min/1 73 m² BSA or more (vide infra) $T^c_{H_2O}$ tends to decrease with an extremely large diuresis of 15–20 ml/min or more (5)

Fig 1 shows the normal variation in patients examined by Whitten & Younes (14) which conforms with the results in other investigations (1 4 5 11 16) Based upon the results reported in the literature values of $T^c_{H_2O}$ max below 3 2 ml/min/1 73 m² BS A must be considered abnormal In isostenuria Cosm and diuresis are equally large and $T^c_{H_2O} = 0$ (baseline) $T^c_{H_2O}$ appears as the horizontal distance between the base line and the patient s curve and the greater the distance the larger the $T^c_{H_2O}$ When $T^c_{H_2O} > 3$ 2 ml/min the patient s curve will fall in the shaded normal area If the curve falls between this area and the base line $T^c_{H_2O}$ is pathologically low

Investigations of this kind have been carried out on adults without kidney disease on adults with acute and chronic renal failure and on patients in the early post operative period (2 4 5 6 10 11 12 13 16) Rapoport *et al* (12) have examined children 8–15 years of age without renal disease Whitten & Younes have done a comparative study of 12 healthy children and 15 with sickle cell anemia all within the 1–15 year age group Hatch *et al* (7) and Perillie *et al* (9) have also described investigations in patients with sickle cell anemia

We have been unable to find any other work dealing with the renal concentrating ability in adults or in children with recurrent urinary infections as evaluated by Cosm and $T^c_{H_2O}$ The results of our investigation however correlate well with similar studies carried out on patients with other diseases Our material is too small for definite conclusions but the results indicate that the method is helpful in detecting impaired renal concentrating capacity at an early stage A moderately depressed concentrating capacity in patients with normal maximum urinary concentration may possibly be revealed by this method However the most important application of this method may be in the cases where the conventional concentrating test is inconclusive Here estimation of Cosm and $T^c_{H_2O}$ may be of value

SUMMARY

In an original study the renal concentrating capacity in ten children with recurrent urinary infections and in one patient with hypospadia has been estimated by maximum urinary specific gravity followed immediately by calculation of Cosm and $T^c_{H_2O}$ during mannitol infusion The normal values found here correlate well with those reported in the literature The results indicate that estimation of Cosm and $T^c_{H_2O}$ may reveal impaired renal concentration capacity at an earlier stage than does the conventional test of maximum urinary specific gravity The method

Cosm $\frac{\text{Urin osm}}{\text{Serum osm}}$ urinvol

T_cH_2O Cosm urinvol

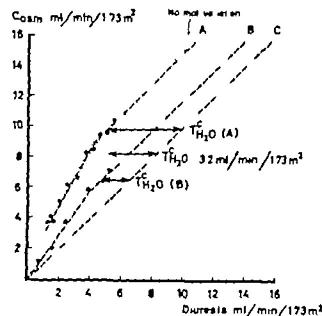


Fig 1 The area of normal variation of $T^c_{H_2O}$ according to Whitten & Younes The A line is based upon the values from patient no 4 as an example of the normals The B line refers to patient no 6 with pathologic value for T_{H_2O} *but no isostenuria The C line represents the isostenuric* patients (zero line)

Calculations The mannitol dosage was adjusted accord ding ot the body weight All other calculations were ad justed to body surface area (BSA) of 1 73 m²

RESULTS

Four patients showed normal values for all para meters Four patients with definite reduction in urinary concentrating capacity revealed pathological $T^c_{H_2O}$ values Two patients showed inconclusive levels of maximum urinary concentration In these two patients the $T^c_{H_2O}$ slope was obviously subnormal however suggesting that the concentrating capacity was in fact affected The hypospadic patient had normal $T^c_{H_2O}$ values These results are shown in Table 1 Table 2 shows the actual variations in serum sodium serum osmolality, urinary osmolality diuresis and Cosm throughout the investigation The $T^c_{H_2O}$ slopes plotted in Fig 1 represent the three groups of patients normal isostenuric and intermediate as well as the area of normal variation

DISCUSSION

During *mannitol* induced osmotic diuresis the tubular reabsorption of water ($T^c_{H_2O}$) increases with the increasing diuresis When the diuresis exceeds a critical value the slope for $T^c_{H_2O}$ will tend to parallel the isostenuric baseline This indicates that $T^c_{H_2O}$ has become stable and the value obtained is called $T^c_{H_2O}$ max This value varies from patient to patient and may also vary in certain patients from one examination to the

Table 1 *Mean $T^c_{H_2O}$ for the individual patients correlated to maximum urinary concentration and other laboratory investigations as well as to the clinical course*

No	Age year	History	X ray	Isotope renogram	Creat/s mg/100 ml	Max urine concent mOsm/l	Level of $T^c_{H_2O}$ max ml/m/1 73 m²
1	3	N	N	0	0	720	3 7
2	5	(+)	N	N	0 7	810	3 6
3	5	–	(P)	N	0 8	980	5 4
4	7	+	P	N	0 8	970	4 3
5	5	–	PP	N	1 0	1010	3 4
6	2	+	P	N	0 8	660	2 6
7	3	+	PP	P	0 8	750	0 6
8	4	+	(P)	O	0 8	540	1 8
9	2	+ +	PP	P	1 5	400	1 6
10	5	+ +	PP	P	0 8	320	0 0
11	13	+ +	PP	N	2 0	320	0 0

N = normal (P) P PP = pathological *findings of increasing severity*
0 = not performed
– = no clinical symptoms when on therapy with antibacterial drug
(+) + + + = relapses of acute infections of increasing severity during therapy

Acta Paediat Scand 59 517–522 1970

SALICYLATE INDUCED FOETAL DAMAGE DURING LATE PREGNANCY IN MICE

The modifying effect of repeated administration and dosage

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For many years teratological experiments have been based on the well known fact that gross malformations can be induced by exogenous factors during organogenesis (2). Thus the early tests for safety of new drugs were designed in agreement with this fact (27). Attention is now being paid to the vulnerability of the foetus in the perinatal period and special recommendations regarding this point have recently been included in the new guidelines (13, 24). There are however only a few experimental reports available. Earlier studies from our laboratory have shown salicylate to produce a variety of foetal injuries when administered late in pregnancy (8, 9, 10, 18).

There has been increasing interest in perinatal pharmacology during the last decade (7). Experience from investigations of drug metabolism late in pregnancy will be of great value for the design of perinatal drug tests. The present study was undertaken to investigate the role of dosage and the frequency of administration on the prenatal effect produced by salicylate. During this study special interest was devoted to the possibility of simultaneously influencing drug metabolism and the foetal damaging effect.

MATERIAL AND METHODS

A lax mice inbred in our laboratory have been used and kept as described earlier (16). Drugs were administered at 10 a.m. in a volume of 0.1 ml distilled water per 20 g body weight, sodium salicylate as i.m. and pentobarbital as i.p. injections.

The LD 50 for sodium salicylate was determined in 25 adult virgin females. They were given different doses between 12 and 18 mg per 20 g body weight. Statistical calculation was made according to the method described by Litchfield & Wilcoxon (19).

The effect of different salicylate-doses on the foetus late in pregnancy was examined in 40 primiparous mice (Table 1). Sodium salicylate was given as single injections in a dose of 3 mg, 10 mg or 15 mg/20 g on day 17 of gestation. The day on which vaginal plug was observed is denoted as day zero of pregnancy. Untreated animals of the same gestational age served as controls. The females were observed daily for premature delivery or maternal death. On day 18 at 10 a.m. undelivered females were sacrificed and the foetuses removed for examination. The number of living and dead foetuses and the developmental stage of the resorbed ones were recorded. Foetal resorptions which had evidently occurred before the mother had received the injection are not included in the tables. Living foetuses were examined macroscopically for gross malformations and for superficial and intra abdominal haemorrhage as described earlier (9, 17).

The effect of single and repeated administration of salicylate was compared in 80 animals (Table 2). Three groups were given sodium salicylate as a single dose of 10 mg/20 g on gestation day 15, 16 or 17. A fourth group received 10 mg/20 g on day 15, 16 and 17. Another group was injected with 3 mg/20 g on day 15 and 16 of gestation and 10 mg/20 g on day 17. The observations of foetal damage were made as described in the dose experiment above but foetal resorption which had occurred before the earliest injection i.e. day 15 is not included.

The effect of salicylate after pretreatment with pentobarbital was studied in 30 animals (Table 3). One group of 20 was given pentobarbital (Nembu

therefore seems valuable as a supplement to the conventional test in selected cases where early detection of beginning deterioration of the renal concentrating capacity is important

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REFERENCES

1 Alken C E Dix V W Weyrauch H M & Wildbolz E *Handbuch der Urologie* Springer Verlag Berlin 1965 vol II p 236
2 Barry K G Cohen A & LeBlanc P Mannitolization I The prevention and therapy of oliguria associated with cross clamping of abdominal aorta *Surgery 50* 353 1961
3 Edelman C M Jr Barnett H L Stark H Boichis K & Soriano J R A standardized test of renal concentrating capacity in children *Amer J Dis Child 114* 639 1967
4 Eisner G M Porush J G Goldstein M R & Levitt M F An appraisal of free water reabsorption in man *N Y Mt Sinai Hosp J 29* 38 1962
5 Goldberg M McCurdy D K & Ramirez M A Differences between saline and mannitol diuresis in hydropenic man *J Clin Invest 44* 182 1965
6 Hallwachs O Klinische Untersuchungen über die Mannitoldiurese an Gesunden und postoperativen Patienten *Klin Wschr 43* 546 1965
7 Hatch F E Culbertson J W & Diggs L W Nature of the renal concentrating defect in sickle cell anemia *J Clin Invest 46* 336 1967
8 Miles B E Paton A & deWardener H E Maximum urine concentration *Brit Med J II* 901 1954
9 Perillie P E & Epstein F H Sickling phenomenon produced by hypertonic solutions A possible explanation of the hyposthenuria of sicklemia *J Clin Invest 42* 570 1963
10 Porush J B & Abramson R G The effect of *hypertonic mannitol on renal sodium excretion in* hydropenic man *Clin Sci 29* 475 1965
11 Rabinowitz L G Au W Y W & Scheer R L Studies on the renal concentrating mechanism IV Osmotic diuresis *J Clin Invest 38* 1725 1959
12 Rapoport S Brodsky W A West C D & Mackler B Urinary flow and excretion of solutes during osmotic diuresis in hydropenic man *Amer J Physiol 156* 433 1949
13 Scheler F Quellhorst E Höffler D & Wigger W Beeinflussung der Nierenfunktion durch Mannitinfusion bei akuter und chronischer Niereninsuffizienz *Schweiz Med Wschr 95 II* 1133 1965
14 Whitten C F & Younes A A A comparative study of renal concentrating ability in children with sickle cell anemia and in normal children *J Lab Clin Med 55* 400 1960
15 Winberg J Determination of renal concentrating capacity in infants and children without renal disease *Acta Paediat Scand 48* 318 1959
16 Zak G A Brun C & Smith H W The mechanism of formation of osmotically concentrated urine during the antidiuretic state *J Clin Invest 33* 1064 1954

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Table 3 *Effect of pentobarbital pretreatment on sodium salicylate induced foetal damage late in pregnancy*

Gestation days injected	Substance and dose per 20 g	No of litters	No of foetuses	Foetal mortality No	%	Superficial haemorrhage in living foetuses No	%	Liver haemorrhage in living foetuses No	%	Gastric haemorrhage in living foetuses No	%
15 16	Pentobarbital 1 5 mg										
17	Salicylate 10 mg	20	147	45	31	27	26	9	9	38	37
15 16	Pentobarbital 1.5 mg	10	83	4	5	1	1	0	0	1	1

tures Four of the ten females given 15 mg/20 g died within 24 hrs after injection Five of the remaining six gave birth before sacrifice In on litter all foetuses were dead

The effect of single and repeated administration of salicylate was compared and showed that repeated injections did not increase the incidence of foetal damage (Table 2) The injection of 10 mg/20 g salicylate on gestation days 15 16 and 17 resulted in the death of 47% of the foetuses When the same dose of salicylate was given on only one of the days *15 16 or 17* foetal death occurred in 39% 55% and 61% of the foetuses respectively However a dose of 3 mg salicylate/20 g on day 15 and 16 followed by 10 mg/20 g on day 17 gave a foetal death incidence of only 21% The difference in incidence of foetal death between this group and the group given 10 mg/20 g only on day 17 was highly significant ($p<0\ 001$) Subcutaneous subcapsular liver and submucosal stomach haemorrhage was found in living foetuses from all groups except that treated only on day 15 (Table 2) Vessel anomalies of the type described earlier (17) were found after injection on day 15

Pretreatment with pentobarbitol in narcotic doses on gestation day 15 and 16 followed by salicylate treatment of 10 mg/20 g on day 17 also diminished the foetal damaging effect exerted by salicylate given as a single dose (Tables 2 and 3) Pretreatment with pentobarbital resulted in a foetal death rate of 31% compared to 61% when salicylate was given alone The difference is highly significant ($p<0\ 001$) The group receiving only pentobarbital on days 15 and 16 showed almost the same death rate (i e 5%) as the uninjected control group (i e 1%) (Tables 1 and 3) As in the other experimental groups superficial liver and gastric haemorrhages were found in some living foetuses (Table 3)

Maternal liver microsomal hydroxylating enzyme for aminopyrine demethylation was increased by pentobarbital but not by salicylate administration (Table 4) No change was ob-

Table 4 *Effect of drug pretreatment on liver microsomal hydroxylating enzymes in adult mouse on day 17 of gestation*

No of animals	Pretreatment	Aminopyrine Demethylation mμmoles FA mg protein/min	NADPH-cytochrome c reductase μmoles NADPH ox/min/mg protein	Cytochrome P-450 ΔE 450-490 mμ/mg protein
	—	7 85	0 095	0 003
	Pentobarbital 1 5 mg/20 g	6 84	0 099	0 011
2	day 15 and 16	14 82	0 066	0 011
	Salicylate 3 mg/20 g	17 48	0 120	0 012
2	day 15 and 16	4 96	0 131	0 012
	Salicylate 10 mg/20 g	8 48	0 118	0 013
	day 15 and 16	7 45	0 111	0 011
		6 78	0 116	0 009

Table 1 *Effect of different doses on sodium salicylate induced foetal damage on day 17 of gestation*

Dose of salicylate per 20 g	No of litters	No of foetuses	Foetal mortality No		Superficial haemorrhage in living foetuses No		Liver haemorrhage in living foetuses No		Gastric haemorrhage in living foetuses No	
—	10	74	1	1	0	0	0	0	0	0
3 mg	10	76	3	4	0	0	1	1	0	0
10 mg	10	77	54	70	9	39	3	13	5	22
15 mg	10[a]	9	9	100						

[a] Four females died within 24 hrs after injection Five delivered before dissection

tal®) 1 5 mg/20 g on gestation day 15 and 16 followed by a 10 mg/20 g salicylate injection on day 17 of gestation A group of 10 was given only pentobarbital in the same dosage The observations of foetal damage were made as described above

The influence of salicylate and pentobarbital pretreatment on maternal liver microsomal hydroxylating enzymes was studied in 8 animals on day 17 of gestation (Table 4) Two animals were treated with pentobarbital 1 5 mg/20 g two animals with 3 mg/20 g salicylate and two others with 10 mg/20 g Two untreated animals of the same gestational age served as controls Liver microsomes were isolated as described by Ernstor *et al* (11) Protein was measured according to the method of Lowry *et al* (20) The oxidative demethylation of aminopyrine and NADPH-cytochrome *c* reductase as well as the amount of cytochrome P 450 in the liver microsomes were assayed as previously described (6 23)

The author is rately indebted to Dr Sten Orrenius and his staff for helping with this part of the study

RESULTS

LD 50 for sodium salicylate in adult virgin females of the A/Jax strain was found to be 15 2 mg/20 g body weight when given as a single i m injection in 0 1 ml distilled water Confidence limits 14 4–16 2 (at $p = 0.05$)

The study of the effect of different do ts of salicylate on the foetus late in pregnancy showed that 3 mg/20 g produced little or no foetal damage (Table 1) After the injection of 10 mg/20 g foetal death was found in 70% Subcutaneous and subcapsular liver haemorrhages of a type described earlier (9) were found in living foetuses at incidences of 39 % and 13% respectively Moreover macroscopically visible submucosal haemorrhage of the stomach was seen in 22% of the foe

Table 2 *Effect of single and repeated administration on sodium salicylate induced foetal damage late in pregnancy*

Gestation days injected	Dose of salicylate per 20 g	No of litters	No of foetuses	Foetal mortality No		Superficial haemorrhage in living foetuses No		Liver haemorrhage in living foetuses No		Gastric haemorrhage in living foetuses No		Vessel anomalies in living foetuses No	
15	10 mg	10	74	29	39	1	2	0	0	0	0	12	27
16	10 mg	10[a]	58	32	55	11	42	0	0	0	0	0	0
17	10 mg	20[b,d]	134	82	61	14	27	6	12	16	31	0	0
15 16 17	10 mg	20	152	72	47	3	4	4	5	7	9	26	33
15 16 17	3 mg 10 mg	20	190	32	21	4	3	14	[illegible]	43	36	0	0

[a] 1 delivered before dissection
[b] 2 delivered before dissection
[c] 1 delivered before dissection
[d] 10 of these are the same ones as in the dose experiment

SUMMARY

The LD 50 for sodium salicylate in adult A/Jax mice has been determined and was found to be 15.2 mg/20 g. Three different doses (15 mg, 10 mg and 3 mg/20 g) have been tested for their foetal damaging effect on day 17 of gestation. The 15 mg dose produced maternal and foetal death or premature delivery. Different types of foetal damage such as foetal death and foetal haemorrhages were found with the 10 mg dose. The 3 mg dose had little or no effect on the foetus.

Sodium salicylate in a dose of 10 mg/20 g was given on days 15, 16 and 17 and compared to a group given salicylate only on day 17. Foetal death was reduced in the group given repeated injections. Sodium salicylate in a dose of 3 mg/20 g was given on days 15 and 16 followed by 10 mg/20 g on day 17. Compared to the group given salicylate 10 mg/20 g only on day 17, there was a highly significant decrease in foetal mortality. Pentobarbital in a narcotic dose of 1.5 mg/20 g was given on day 15 and 16 of gestation followed by salicylate 10 mg/20 g on day 17. Foetal mortality was significantly reduced when compared with the group treated with a single salicylate injection of 10 mg/20 g on day 17 of gestation.

Induction of hydroxylating enzymes was studied in a few animals. In the adult day 17 pregnant female pentobarbital, but not salicylate treatment gave increased enzyme activity.

The results are discussed with reference to the design of teratology tests. Furthermore the mechanisms behind the modifying effect of repeated drug administration are debated.

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REFERENCES

1. Arias, I. M., Gartner, L., Furman, M. & Wolfson, S. Effect of several drugs and chemicals on hepatic glucuronide formation in newborn rats. *Proc Soc Exp Biol Med 112*: 1037, 1963.
2. Baker, J. B. E. The effects of drugs on the foetus. *Pharmacol Rev 12*: 37, 1960.
3. Burns, J. J., Cucinell, S. A., Koster, R. & Conney, A. H. Application of drug metabolism to drug toxicity studies. *Ann N Y Acad Sci 123*: 273, 1965.
4. Catz, C. & Yaffe, S. J. Barbiturate enhancement of bilirubin conjugation and excretion in young and adult animals. *Pediat Res 2*: 361, 1968.
5. Conney, A. H. Pharmacological implications of microsomal enzyme induction. *Pharmacol Rev 19*: 317, 1967.
6. Dallner, G. Studies on the structural and enzymic organization of the membranous elements of liver microsomes. *Acta Path Microbiol Scand Suppl 166*, 1963.
7. Done, A. K. Perinatal pharmacology. *Ann Rev Pharmacol 6*: 189, 1966.
8. Eriksson, M. Effect of salicylate treatment on fetal and maternal prothrombin time in the mouse. *Acta Physiol Scand 78*: 39, 1970.
9. — Salicylate induced foetal haemorrhage in two mouse strains. *Acta Path Microbiol Scand 76*: 164, 1969.
10. Eriksson, M. & Larsson, K. S. Premature birth induced in mice by salicylate. *Nature 220*: 385, 1968.
11. Ernster, L., Siekevitz, P. & Palade, G. E. Enzyme-structure relationships in the endoplasmic reticulum of rat liver. *J Cell Biol 15*: 541, 1962.
12. Franke, H. & Klinger, W. Untersuchungen zum Mechanismus der Enzyminduktion IX. Die Wirkung von Barbital auf die Substruktur der Leberzellen gravider Ratten und ihrer Foeten. *Acta Biol Med German 17*: 507, 1966.
13. Guidelines for reproduction studies for safety evaluation of drugs for human use. FDA, USA, 1966.
14. Hart, L. G., Adamson, R. H., Dixon, R. L. & Fouts, J. R. Stimulation of hepatic microsomal drug metabolism in the newborn and fetal rabbit. *J Pharmacol Exp Ther 137*: 103, 1962.
15. Hanninen, O. Effect of salicylamide administration on D-glucuronic acid metabolism in the rat. *Ann Acad Sci Fenn (Med) 123*: 1, 1966.
16. Larsson, K. S. Studies on the closure of the secondary palate. III. Autoradiographic and histochemical studies in the normal mouse embryo. *Acta Morph Neerl Scand 4*: 349, 1962.
17. Larsson, K. S., Boström, H. & Ericson, B. Sali

served for NADPH-cytochrome c reductase or cytochrome P-450 The values are calculated in relation to the protein content

DISCUSSION

The most interesting finding in this investigation is the protection against the foetal damaging effect of a single high dose of salicylate by pretreatment with small doses of salicylate It is not known if salicylate pretreatment acts via the mother the foetus or both Certain drugs even in small doses, have been shown to increase the level of liver microsomal enzymes with a concomitant faster detoxification of drugs (5) Human newborns of phenobarbitone treated mothers have greater capacity to conjugate bilirubin (21) Moreover in animal experiments, phenobarbital and benzpyrene have been shown to increase the activity of liver enzymes in the foetus and newborn when given to the mother late in pregnancy (1 4 14) This observation has not been substantiated in morphological studies of smooth surfaced endoplasmic reticulum (12) However this induction of enzyme activity can only take place in the foetus during the very last days of gestation (14)

Barbituric acid derivatives are the most common inducers of enzyme activity (4 12) In this study pretreatment with pentobarbital had about the same protective effect against foetal damage as pretreatment with small doses of salicylate This suggests that drug induced enhancement of detoxification of salicylate could be one explanation for the protective effect of both pentobarbital and salicylate The results from the biochemical studies on liver microsomal enzyme induction do not support this assumption However the activities investigated in the present study did not include glucuronide and glycine formation which are the most important pathways of salicylate detoxification (25 26) There are diverging reports in the literature on the effect of salicylate on its own metabolism On the one hand repeated salicylamide administration to rats has been shown to decrease its toxicity and increase the urinary output of glucuronic acid (15) On the other hand, chronic administration of acetylsalicylic acid to dogs does not cause a decrease in its serum concentration (3)

The current recommendations for reproductive studies for safety evaluation of drugs for human use now include perinatal studies (13 24) Concerning the doses and the method of administration there are no differences from the studies in the organogenetic period Thus it is recommended to use at least two doses which should be selected with reference to the dose response of the adult animal In this study 15 mg/20 g a dose close to LD 50 (15 2 mg/20 g) was chosen for comparison to the empirically found teratogenic dose of 10 mg/20 g (17) The lethal effect of the high dose in pregnant animals was of the same magnitude as in non pregnant females Premature birth has been regarded as an expression of foetal damage of salicylate treatment late in pregnancy (10) This was also found in the present study but only with the highest dose used Other types of foetal damage, such as haemorrhages and death were found with the 10 mg dose chosen because of its empirically established teratogenic effect (17) It is interesting to note that there is a very small range between the LD 50 for adult mice and the potent foetal damaging dose Studies on tumour inhibiting chemicals have been made earlier in which LD 50 and the teratogenic dose were compared and the range was shown to vary considerably (22)

In teratological experiments drugs are sometimes given on only one day of gestation but more commonly administered throughout pregnancy This study has shown however that continuous administration of a drug even for a short period is able to modify the teratogenic response

The results of the present investigation support the need for further pharmacological studies in the safety evaluation of drugs

SUMMARY

›e LD 50 for sodium salicylate in adult /Jax mice has been determined and was ›und to be 15 2 mg/20 g Three different ›ses (15 mg 10 mg and 3 mg/20 g) have ›en tested for their foetal damaging effect on ›y 17 of gestation The 15 mg dose produced ›aternal and foetal death or premature de ›very Different types of foetal damage such ›s foetal death and foetal haemorrhages were ›ound with the 10 mg dose The 3 mg dose ›ad little or no effect on the foetus

Sodium salicylate in a dose of 10 mg/20 g ›as given on days 15 16 and 17 and com ›ared to a group given salicylate only on day 17 Foetal death was reduced in the group given repeated injections Sodium salicylate in a dose of 3 mg/20 g was given on days 15 and 16 followed by 10 mg/20 g on day 17 Compared to the group given salicylate 10 mg/20 g only on day 17 there was a highly significant decrease in foetal mortality Pentobarbital in a narcotic dose of 1 5 mg/20 g was given on day 15 and 16 of gestation followed by salicylate 10 mg/20 g on day 17 Foetal mortality was significantly reduced when compared with the group treated with a single salicylate in jection of 10 mg/20 g on day 17 of gestation

Induction of hydroxylating enzymes was studied in a few animals In the adult day 17 pregnant female pentobarbital but not salicy late treatment gave increased enzyme activi ty

The results are discussed with reference to the design of teratology tests Furthermore the mechanisms behind the modifying effect of re peated drug administration are debated

ACKNOWLEDGEMENTS

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REFERENCES

1 Arias I M Gartner L Furman M & Wolfson S Effect of several drugs and chemicals on he patic glucuronide formation in newborn rats *Proc Soc Exp Biol Med 112* 1037 1963

2 Baker J B E The effects of drugs on the foe tus *Pharmacol Rev 12* 37 1960

3 Burns J J Conney S A Koster R & Con ney A H Application of drug metabolism to drug toxicity studies *Ann N Y Acad Sci 123* 273 1965

4 Catz, C & Yaffe S J Barbiturate enhancement of bilirubin conjugation and excretion in young and adult animals *Pediat Res* 2 361 1968

5 Conney A H Pharmacological implications of microsomal enzyme induction *Pharmacol Rev 19* 317 1967

6 Dallner G Studies on the structural and en zymic organization of the membranous elements of liver microsomes *Acta Path Microbiol Scand* Suppl *166* 1963

7 Done A K Perinatal pharmacology *Ann Rev Pharmacol 6* 189 1966

8 Eriksson M Effect of salicylate treatment on fetal and maternal prothrombin time in the mouse *Acta Physiol Scand 78* 39 1970

9 — Salicylate induced foetal haemorrhage in two mouse strains *Acta Path Microbiol Scand 76* 164 1969

10 Eriksson M & Larsson K S Premature birth induced in mice by salicylate *Nature* 220 385 1968

11 Ernster L Siekevitz, P & Palade G E En zymestructure relationships in the endoplasmic reticulum of rat liver *J Cell Biol 15* 541 1962

12 Franke H & Klinger W Untersuchungen zum Mechanismus der Enzyminduktion IX Die Wir kung von Barbital auf die Substruktur der Leber zellen gravider Ratten und ihrer Foeten *Acta Biol Med German 17* 507 1966

13 Guidelines for reproduction studies for safety evaluation of drugs for human use FDA USA 1966

14 Hart, L G Adamson R H, Dixon, R L & Fouts, J R Stimulation of hepatic micro somal drug metabolism in the newborn and fetal rabbit *J Pharmacol Exp Ther 137* 103 1962

15 Hartiala, O Effect of salicylamide administra tion on D-glucuronic acid metabolism in the rat *Ann Acad Sci Fenn (Med)* 1 3 1 1964

16 Larsson, K S Studies on the closure of the secondary palate III Autoradiographic and histochemical studies in the normal mouse em bryo *Acta Morph Neerl Scand 4* 349 196..

17 Larsson K S., Boström H & Ericson, B Sali

cylate induced malformations in mouse embryos *Acta Paediat Scand 52* 36 1963

18 Larsson K S & Eriksson M Salicylate induced fetal death and malformations in two mouse strains *Acta Paediat Scand 55* 569 1966

19 Litchfield J T & Wilcoxon F A simplified method of evaluating dose-effect experiments *J Pharmacol Exp Ther 96 99 1949*

20 Lowry O H Rosebrough N J Farr A L & Randall R J Protein measurement with the Folin phenol reagent *J Biol Chem 193* 265 1951

21 Maurer H M Wolff J A Finster M Poppers P J Pantuck E Kuntzman R & Conney A H Reduction in concentration of total serum bilirubin in offspring of women treated with phenobarbitone during pregnancy *Lancet II* 122 1968

22 Murphy M L Teratogenic effects of tumour inhibiting chemicals in the foetal rat *In* G E W Wolstenholme & C M O Connor (eds) *Ciba Foundation Symposium on Congenital Malformations* J & A Churchill London 1960 p 78

23 Orrenius S & Thor H Fatty acid interaction with the hydroxylating enzyme system of rat liver microsomes *Europ J Biochem 9* 415 1969

24 Report of a WHO Scientific Group Principles for the testing of drugs for teratogenicity *Pharm Ind 29* 759 1967

25 Smith M J H & Smith P K *The Salicylates* John Wiley & Sons New York 1966

26 Sturman J A Dawkins P D McArthur K & Smith M J H The distribution of salicylate in mouse tissues after intraperitoneal injection *J Pharm Pharmacol 20* 58 1968

27 Tuchmann Duplessis H Design and interpretation of teratogenic tests *in* J M Robson F Sullivan & R L Smith (eds) *Biological Council Symposium on Embryopathic Activity of Drugs* J & A Churchill London 1965 p 56

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DIAGNOSIS OF BETA THALASSAEMIA IN THE NEWBORN BY MEANS OF HAEMOGLOBIN SYNTHESIS

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It is now well established that the most significant metabolic defect in beta Thalassaemia is a decreased ability of erythroid cells to synthesize beta globin chains resulting in a defective production of Hb A with a relative excess of alpha chains synthesis The defect in beta chains synthesis can be partial (2 7 12) or complete (3 5 10)

These abnormalities can be detected in the erythroid cells from both peripheral blood (2 3 7 10 12) and bone marrow (5) A prominent role in the genesis of the thalassaemic anaemia has been ascribed to the excess in alpha chains synthesis (2) and a strict correlation between the rate of excess alpha chain synthesis and the reduced survival of thalassaemic erythrocytes labelled with radioactive chromate and transfused into healthy group compatible recipients has been demonstrated (10)

Since the main metabolic defect in beta Thalassaemia is genetically determined it should be present at birth because both normal newborn and premature infants synthesize consistent amounts of beta globin chains The demonstration at birth of a disturbance in haemoglobin synthesis due to defective beta chains production would enable early diagnosis of beta Thalassaemia

Such early detection would be important not only in relation to the possibility of prompt supportive therapy or a future specific treatment of the disease but also permit studies of the intrinsic metabolic defect before the onset of secondary factors It would also enable a description of the development of secondary factors which contribute to create the clinical pattern observed in these patients at a later stage of the disease

With this purpose in mind we have carried out a study of haemoglobin synthesis in three newborns whose parents are carriers of the beta Thalassaemia trait

SUBJECTS AND METHODS

Subjects studied

Three newborns (cases 1 2 and 3) whose parents are heterozygotes for the beta Thalassaemia gene have been studied At 3 days and (in cases 1 and 2) at 2 4 and 5 months of age the following investigations were performed haemoglobin synthesis, haemoglobin electrophoresis both by starch gel and cellulose acetate membrane determination of alkali resistant haemoglobin red blood cell osmotic resistance and complete blood counts

A control study of haemoglobin synthesis was performed in two premature newborns (cases 4 and 5) whose parents had no haematological abnormalities and in two children aged 2 and 7 years (cases 6 and 7) with reticulocytosis secondary to bleeding anaemia.

Clinical data

Case 1 (C. Z.) Born at term Parents heterozygotes for beta Thalassaemia gene Elder brother affected by beta Thalassaemia major

The pregnancy and the delivery were uneventful.

Table 1 *Haematological data of cases 1 2 and 3*

Case	Age	Hb (g)	R B C (mm³)	Erythroblasts ( )	Reticulocytes (‰)	Hb A_2 ( )	Alkali resistant Hb ( )	Osmotic fragility test NaCl Min	Med	Max
1 C Z	3 days	15 8	5 150 000	4	30	—	94	0 46	0 34	0 21
	2 mo	12 8	4 310 000	0	29	1	58	0 46	0 32	0 28
	4 mo	12 5	4 100 000	0	25	1 6	30	0 45	0 31	0 28
	5 mo	12 0	3 800 000	0	15	2	—	0 44	0 34	0 26
2 S S	3 days	16 0	5 300 000	3	35	—	100	0 48	0 34	0 28
	2 mo	12 6	4 290 000	2	49	1 7	98	0 45	0 32	0 26
	4 mo	9 6	3 650 000	2	50	2 5	98	0 45	0 31	0 26
	5 mo	6 0	2,600 000	9	50	2 6	97	0 44	0 30	0 24
3 S C	2 days	13 2	5 050 000	5	32	—	100	0 48	0 33	0 21
	2 mo	4 0	1 800 000	10	60	2 2	97	0 44	0 30	0 23

Birth weight was 2750 g He has always been in good health When last examined (12 months) the clinical and haematological examinations were in the normal range

Case 2 (S S) Born at term Parents bear the beta Thalassaemia trait Birth weight was 2700 g The clinical examination at the third day of life was normal

She remained well and the growth and development continued normally till the fifth month when hypochromic anaemia and splenomegaly were detected The clinical and haematological pattern of beta Thalassaemia major is now clear and transfusion treatment has been started

Case 3 (S C) Born at term Parents are heterozygotes for the beta Thalassaemia gene Three siblings are affected by beta Thalassaemia major and one is normal The pregnancy and the delivery were uneventful Birth weight was 3300 g Neonatal examination revealed no clinical signs except mild jaundice which became evident on the third day of life and disappeared in a few days

She remained well until the second month of life when hypochromic anaemia and hepatomegaly without splenomegaly was noted At this age clinical observation and haematological laboratory data were typical for beta Thalassaemia major

Cases 4 and 5 were prematurely born after uneventful pregnancies of 33 and 31 weeks respectively At the present time they are 14 and 24 months old and in good health The parents bear no stigmata of beta Thalassaemia or other haematological abnormalities

Cases 6 and 7 suffered from mild bleeding anaemias and were selected because of the high reticulocyte count found in the peripheral blood The parents are not carriers of the beta Thalassaemic trait and presented no other haematological abnormalities

The haematological data of subjects 1 2 and 3 are summarized in Table 1

Methods

Routine haematological studies were performed according to the techniques described by Dacie (6)

Fetal haemoglobin percentages were determined by the alkali denaturation method of Singer et al (9)

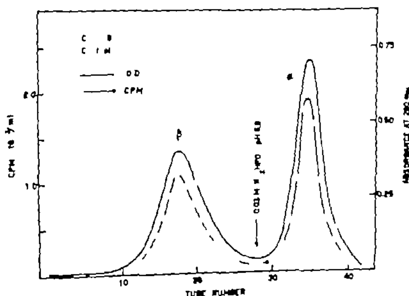


Fig 1 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 6 (bleeding anaemia) after incubation of the erythrocytes with tritiated amino acids

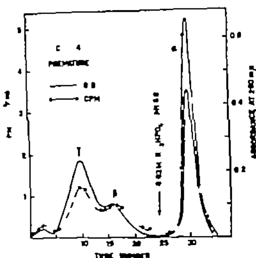


Fig 2 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 4 (premature) after incubation of the erythrocytes with tritiated amino acids

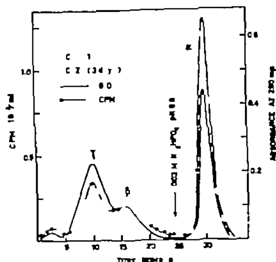


Fig 3 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 1 (C Z) after incubation of the erythrocytes with tritiated amino acids

Starch gel and cellulose acetate membrane electrophoresis were performed using the methods described by Huisman (8) and Briere (4) respectively

Haemoglobin synthesis was studied by incubating samples of the peripheral blood with all the amino-acids required for protein synthesis valine leucine lysine were tritiated Globins prepared by the acid acetone method were subjected to column chromatography on CM cellulose and the eluted peaks continually quantitated with a Gilford automatic recording spectrophotometer the radioactivity incorporated was directly counted in a Tri-carb liquid scintillation counter The detailed technical procedures have been described elsewhere (3) and were followed without modifications Haemoglobin column chromatography on Amberlite IRC 50 was performed following the indications of *Allen et al* (1) All blood samples used for study were collected by venipuncture in heparinized syringes from fasting subjects and used immediately

RESULTS

In the two control subjects (cases 6 and 7) haemoglobin synthesis was normal The elution profile after chromatography of the labelled

Table 2 [³H] *amino acid (leucine valine lysine) incorporation into α β γ and δ globin chains in three newborns whose parents are heterozygotes for the β thalassaemia gene*

Case	Age	Radioactivity*			
		[³H] α		[³H] (β+γ+δ)	[³H] α / [³H] (β+γ+δ)
1 C Z healthy	3 days	9 750		9 600	1 01
	2 mo	8 500		8,500	1 00
	4 mo	6 100		6,200	0 99
	5 mo	5 700		5 800	0 99
		[³H] α	[³H] β	[³H] (γ+δ)	[³H] α / [³H] (γ+δ)
2 S S Thalassaemia major	3 days	49 000	0	24,500	2.0
	2 mo	78 500	0	41 000	1 9
	4 mo	41,500	0	21 800	1 9
	5 mo	48 500	0	25 400	1 9
3 S C Thalassaemia major	2 days	70 700	0	39 000	1 8

*Radioactivity is expressed as counts min incorporated into 0 4 ml of packed red cells

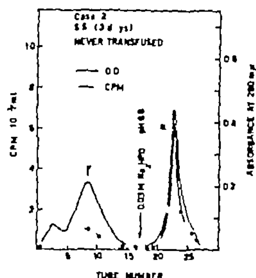


Fig 4 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 2 (S S) after incubation of the erythrocytes with tritiated amino acids

globin chains on CM (Fig 1) reveals the presence of two peaks of proteins corresponding to the beta and alpha globin chains Both peaks were radioactive indicating active synthesis of beta and alpha globin chains The same amount of protein and incorporated radioactivity was present under the two peaks, as found in normal adult subjects ratio alpha/beta + delta = 1 [1] In the two premature infants (cases 4 and 5) the elution pattern after CM chromatography revealed three radioactive protein peaks corresponding to gamma beta and alpha globin chains (Fig 2) The ratio alpha/gamma + beta + delta is equal to 1 both for radioactivity and for protein The high specific activity of the beta chain demonstrates that the change from gamma to beta chain production is under way

In case 1 (C Z) haemoglobin synthesis studied on the third day of life was normal the elution pattern (Fig 3) being identical to that found in the normal newborns and prematures Haemoglobin synthesis studied at the third and fifth months of life showed a gradual substitution of gamma chains by beta chains (Table 2)

In cases 2 and 3 (S S and S C) haemoglobin synthesis studied at birth presented an abnormal chromatographic pattern the elution profile shows complete absence of the peak corresponding to beta globin and an excess synthesis of alpha globin chains over the other globin chains (Figs 4 and 5 and Table 2) resulting in an abnormal [³H] alpha /[³H] (gamma + delta) characteristic of beta thalassaemia major Chromatography on Amberlite IRC 50 (Fig 6) revealed the presence of only haemoglobin F and confirmed the results

DISCUSSION

The clinical course and changes in the haematological data during followup examinations (Table 1) confirmed the results obtained by means of haemoglobin synthesis at birth Case 1 who presented a normal haemoglobin synthesis at birth did not develop any subsequent haematological abnormalities and was healthy at examination at the age of 18 months In cases 2 and 3 who manifested disorders in haemoglobin synthesis at birth characterized by an absence of beta globin chain synthesis

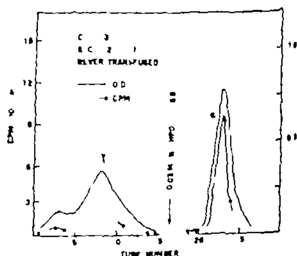


Fig 5 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 3 (S C) after incubation of the erythrocytes with tritiated amino acids

[1] Delta chain cannot be separated with this chromatographic technique Delta chain from purified Hb A_2 is eluted immediately after the beta chain

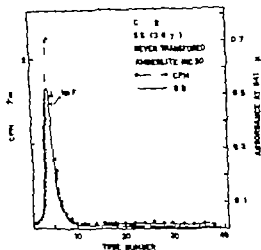


Fig 6 Elution pattern from Amberlite IRC 50 of the labelled haemoglobins of case 2 (S. 8.) after incubation of the erythrocytes with tritiated amino acids

and an excess of alpha globin chain synthesis beta Thalassaemia major has become clinically evident during followup

These findings clearly indicate that the diagnosis of beta Thalassaemia can be confirmed at birth when typical clinical features are absent by demonstrating the existence of the specific metabolic defect of the thalassaemic red cell i e absence of beta globin chain synthesis and excess of alpha globin chain synthesis This can be demonstrated even when other routine haematological studies are normal

This method permits also a neonatal diagnosis of the beta Thalassaemia trait as Weatherall & Nathan have recently demonstrated (11)

It must also be pointed out that if we consider the patterns of haemoglobin synthesis in the prematures this possibility must be extended also to the prenatal period (at least from the seventh month of intrauterine life)

SUMMARY

Haemoglobin synthesis has been studied in three newborns whose parents are carriers of the beta Thalassaemia trait Demonstration of absence of beta globin chain synthesis and excess of alpha globin chain synthesis permitted diagnosis of beta Thalassaemia major in two of three subjects in absence of any other sign of the disease

The clinical course and change in the haematological data during followup examinations confirmed the diagnosis performed at birth In the third subject no disorder in haemoglobin synthesis was ascertained at birth he remained well and is now perfectly healthy On this evidence it is suggested that beta Thalassaemia can be detected at birth in absence of any other sign of the disease

REFERENCES

1 Allen D W Schroeder W A & Balog J Observations on the chromatographic heterogeneity of normal adult and fetal human haemoglobin a study on the effects of crystallization and chromatography on the heterogeneity and isoleucine content *J Amer Chem Soc* *80* 1628 1958

2 Bank A & Marks P A Excess α-chain synthesis relative to β-chain synthesis in Thalassemia major and minor *Nature* *212* 1198 1966

3 Bargellesi A Pontremoli S & Conconi F Absence of β globin synthesis and excess of α globin synthesis in homozygous β Thalassaemia *European J Biochem* *1* 73 1967

4 Briere R O Golias T & Batsakis J G Rapid qualitative and quantitative hemoglobin fractionation *Amer J Clin Path* *44* 695 1965

5 Conconi F Bargellesi A Pontremoli S Vigi V Volpato S & Gaburro D Absence of β globin synthesis and excess of α globin synthesis in homozygous β Thalassaemic subjects from the Ferrara Region *Nature* *217* 259 1968

6 Dacie J V *Manuale di Tecnica ematologica* Piccin ed Padova 1957

7 Heywood, D Karon M & Weissman S, Asymmetrical incorporation of aminoacids in the alpha and beta chains of hemoglobin synthesized by thalassemic reticulocytes *J Lab Clin Med* *66* 4 6 1965

8 Huisman, T H J Normal and abnormal human hemoglobins *Ad vanc Clin Chem* *6* 476 1963

9 Singer K Chernoff A L & Singer L Studies on abnormal hemoglobins their demonstration in sickle cell anemia and other hematological disorders by means of alkali denaturation *Blood* *6* 413 1951

10 Vigi V, Volpato S, Gaburro D Conconi F, Bargellesi, A & Pontremoli S The correlation between red cell survival and excess of α globin

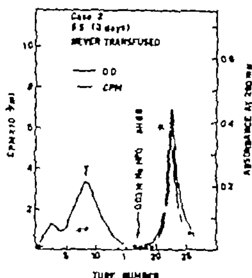


Fig 4 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 2 (S S) after incubation of the erythrocytes with tritiated amino acids

globin chains on CM (Fig 1) reveals the presence of two peaks of proteins corresponding to the beta and alpha globin chains Both peaks were radioactive indicating active synthesis of beta and alpha globin chains The same amount of protein and incorporated radioactivity was present under the two peaks as found in normal adult subjects ratio alpha/beta + delta = 1 [1] In the two premature infants (cases 4 and 5) the elution pattern after CM chromatography revealed three radioactive protein peaks corresponding to gamma, beta and alpha globin chains (Fig 2) The ratio alpha/gamma + beta + delta is equal to 1 both for radioactivity and for protein The high specific activity of the beta chain demonstrates that the change from gamma to beta chain production is under way

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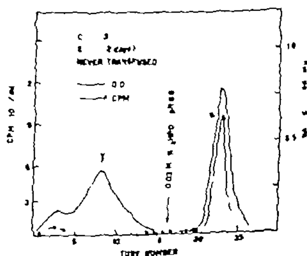


Fig 5 Elution pattern from CM cellulose column chromatography of the labelled globin chains of case 3 (S C) after incubation of the erythrocytes with tritiated amino acids

[1] Delta chain cannot be separated with this chromatographic technique Delta chain from purified Hb A_2 is eluted immediately after the beta chain

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GENETIC STUDIES IN GLYCOGEN STORAGE DISEASE TYPE III

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Recent biochemical advances have resulted in a division of glycogen storage disease (GSD) into at least 7 types with different enzymatic defects (3 7 12 20) It is now also possible to detect mild forms of the disease Type III is of particular interest for genetic studies since a deficiency of the debranching enzyme activity usually can be demonstrated in the blood corpuscles The debranching enzyme system catalyzes the degradation of branching points in glycogen (6)

It is still difficult to obtain definite genetic conclusions in the different types of GSD from the available data in the literature (12) Valuable information were obtained from screening studies of enzyme activity and glycogen content of blood corpuscles from relatives of patients with types III and VI (2 14 17 18 19) Recently Van Hoof (15) demonstrated that the enzymatic defect of erythrocytes of the parents of 14 patients with GSD type III were in most cases intermediary between the normal and pathological situations There was however an important overlapping both with normal and pathological values Moreover Huijing *et al* (6) when studying the activity of the debranching enzyme system in leucocytes were not able to discover heterozygotes among relatives of patients with GSD type III It appears therefore that further investigations of the relatives of patients with GSD type III were of great interest The present paper reports the results of erythrocyte studies performed on samples from a large number of members of four families with this particular type of GSD

The biochemical part of this work was carried out by O O T at the Department of Biochemistry Årstadvollen 19 Bergen Norway

MATERIALS

The four families with type III have been selected from a total of 20 cases of GSD traced in Norway (Table 1) The 20 cases were collected by contacting different departments at the University Hospitals in Norway and by sending an inquiry to all departments of pediatrics and internal medicine in Norway Seven of the cases are from the Department of Pediatrics University of Bergen Further details will be given in a later publication (Moe *et al*) Ten of the cases have been typed as to their specific enzyme deficiency Six of the cases turned out to be of type III These 6 cases as well as 2 cases of type V and 1 case of type VI are from the Western Coast of Norway

Amylo-1 6-glucosidase activity and glycogen content were studied in the erythrocytes from 5 of the

Table 1 *Cases of glycogen storage disease traced in Norway*

GSD type	No of cases	Reported previously: No of cases	Reported previously: Author
II	1	1	Kluge (9)
III	6	1	Høyer & Moe (7)
V	2	2	Skullerud (13)
VI	2	1	Høyer & Moe (7)
Unclassified*	9	0	
Total	20	5	

* Enzyme studies not performed

synthesis in β Thalassaemia *Brit J Haemat 16* 25 1969
11 Wai Kan Y & Nathan D G Beta Thalassaemia Trait Detection at Birth, *Science 161* 389 1968
12 Weatherall D J Clegg J B & Naughton M A Globin synthesis in Thalassaemia an in vitro study *Nature 208* 1061 1965

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Key words Beta thalassaemia, newborns haemoglobin synthesis alfa globin chain synthesis beta globin chain synthesis

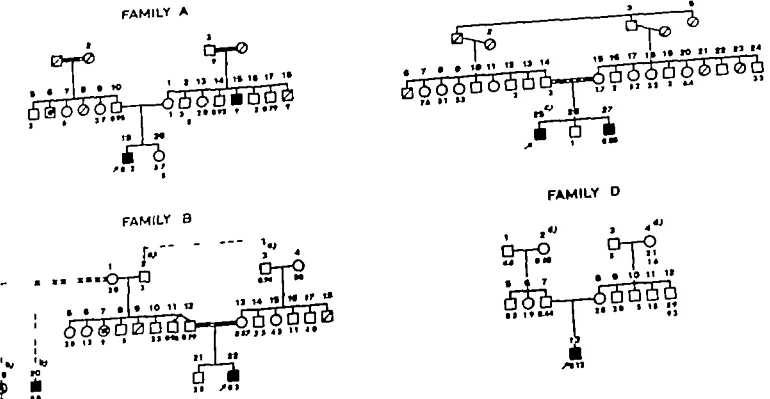


Fig. 1. Pedigrees of 4 families with glycogen storage.

Table 2 *Activity of amylo 1 6 glucosidase in erythrocytes from patients of type III GSD their relatives and healthy controls*

Group	*No of cases*	Range *(units/g* Hb)	Mean *(units/g* Hb)
Patients type III	5	0–0.2	0.097
Parents of patients	*8*	*0.79–2.8*	*1.23*
Grandparents of patients	9	0.62–5.9	2.77
Siblings of parents	37	0.5–7.6	3.06
Siblings of patients	*3*	*1.6–5.7*	*3.36*
Healthy controls	*15*	*1.7–7.3*	*4.10*

patients with type III GSD and a total of 57 of the relatives of the patients The control material consisted of 15 healthy individuals above the age of *10 years Only slightly higher amylo 1 6 glucosidase* activity of erythrocytes was found by Van Hoof (15) in children below 10 years compared to healthy adults It was therefore considered unnecessary by us to include small healthy children in the control material *particularly due to the fact that only 3 of the relatives of the patients were under the age of 10 years*

(U C) glucose was purchased from the Radiochemical Centre Amersham Bucks

Glycogen type II (from shell fish) was obtained from Sigma

DESCRIPTION OF SIX PATIENTS WITH ESTABLISHED TYPE III GSD

Case A was a 28 months old male child with an enlarged liver Biochemical investigations of liver and muscle biopsies (*Professor Hers et al* Louvain) showed high glycogen content in liver and moderately high in muscle The polysaccharide stored in both *tissues had the structure of limit dextrin Complete* lack of amylo 1 6 glucosidase activity was demonstrated Studies of glycogen content and amylo 1 6 glucosidase activity of erythrocytes gave concordant results

Case B_{II} was a 25 years old male with a history of dyspnoea and enlarged liver in childhood He is *now completely healthy with no liver enlargement*

Case B_{III} a 9 months old male infant had a history of neonatal prolonged jaundice Later on the liver was enlarged and he had attacks of convulsions responding *to frequent feedings*

Case C_{II} was a 7 years old male child This case has been published previously (7) Biochemical investigations of *liver biopsy (professor Hers et al* Louvain) showed high glycogen content and complete lack of amylo 1 6 glucosidase activity

Case C_{IV} a 2 years old brother of case C_{II} had attacks of general convulsions during his first year of life He still has an enlarged liver

Case D_{II} was a 21 months old male child with an enlarged liver

In cases B_{II} B_{III} C_{IV} and D_{II} the type diagnosis was made by erythrocyte studies (Fig 1 and Table 4)

METHODS

About 10 ml venous blood specimens were collected on heparin (preserved with chlorocresol 0.15) and *stored at 0 C Within 2 days an erythrocyte suspension* was obtained from the blood specimens as described by Van Hoof (15) The precipitate of washed erythrocytes was resuspended in 1 volume of isotonic NaCl and kept in a deepfreezer until further processed *After thawing the hemolysate was* centrifuged for 15 min at 10 000 × g The supernatant *was poured into an ice cooled test tube and stirred* for a few seconds followed by removal of 4 7 aliquots of 0.2 ml for the determination of amylo-1 6-glucosidase activity Two aliquots of 25 µl were used for the determination of hemoglobin as cyanmet *hemoglobin (11) Aliquots of 2.0 ml were frozen for* the determination of glycogen content

Amylo 1 6 glucosidase activity was assayed by the incorporation of (U C) glucose into glycogen (U) *0.2 ml of hemolysate together with 0.2 ml of a* % (w/v) glycogen solution and 0.05 ml (U C) glucose *(1.5 µC specific activity 1 µC/µmole)* was incubated at various lengths of time up to 4 hours At the end of the incubation 0.5 ml of 1.5 M trichloro*acetic acid and 2 ml of distilled water were added* under continuous agitation followed by centrifugation The glycogen was alchoholprecipitated 3 times before digestion with 2 ml of 20 KOH and a further 3 times afterwards The final precipitate was dissolved in 0.7 ml water of which 0.5 ml was used for the radioactivity determined by liquid scintilla*tion counting The results were expressed* in units per gram of hemoglobin one unit being the amount of enzyme incorporating 0.1% *of the counts added* as glucose in *1 hour (15)*

The glycogen was extracted from hemolysate with cold trichloroacetic acid as described by Van Hoof (15) After dialysis and reduction of the volume *treatment with takamine diazyme was performed and* was followed by the glucose oxidase reaction The glycogen was then calculated

RESULTS

Pedigrees and data concerning the activity of amylo-1 6-glucosidase activity *of the members* of the four families are shown in Fig 1 Table *2 summarizes the results of the determinations* of amylo 1 6 glucosidase activity of erythrocytes in 5 patients the different groups of relatives and the 15 healthy controls *A total of* 22 determinations on different samples *from* the 15 healthy controls were performed No

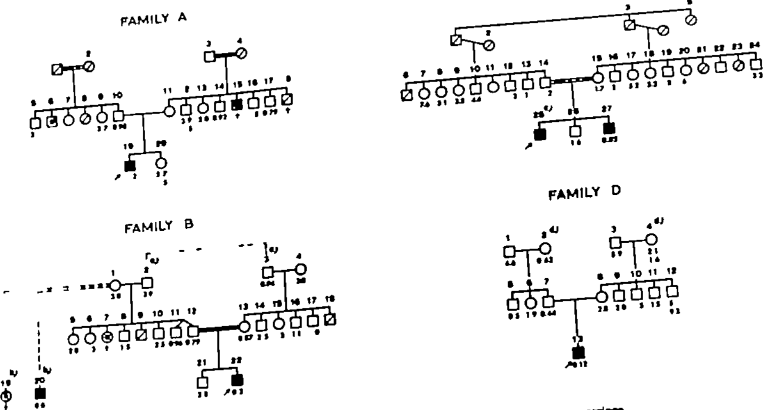


Fig. 1. Pedigrees of 4 families with glycogen storage disease type III.

Table 3 *Amylo 1 6 glucosidase activity of red blood cells before and after a meal in three healthy adults*

Case	After overnight fasting (units/g Hb)	Two hours after a meal (units/g Hb)
GW	5 5	5 7
KT	3 1	3 1
PW	2 5	2 6

difference before and after a meal was found in 3 of the healthy subjects (Table 3) Five determinations were performed in the control subject with the lowest initial value (1 7 units/g Hb) all the subsequent 4 values being higher than the initial The mean of the first determination in each of the 15 controls was 4 10 units/g Hb (Table 1) The distribution of the normal material showed two populations as there was a smaller population of high values A similar observation was made by Van Hoof (15) The mean—2 S D can therefore not be used in calculating the lower border of normal values However, none of the 22 values in the 15 healthy controls were below 1 7 units/g Hb

The mean values of the 5 patients and of their 8 parents were 0 098 and 1 23 units/g Hb respectively There was no overlapping between the two groups Six of the 8 parents had amylo-1 6 glucosidase activity of erythrocytes below 1 3 units/g Hb (Fig 1)

The activity of amylo 1 6 glucosidase of erythrocytes from 20 of a total of 57 relatives of the patients was below 1 7 units/g Hb (Fig 1) Nine of the 20 values were below 1 unit/g Hb

Table 4 *Glycogen concentration of erythrocytes from patients with GSD type III*

Patients[a]	Glycogen in erythrocytes μg/g Hb
A_{II}	311
B_{I}	365
B_{III}	1040
C_{II}	224
D_{II}	264

[a] For identification of the patients see Fig 1

Consanguinity occurred in 3 of the families (A, B and C) Family D was distantly related to family A through two different lines (Fig 1) Table 4 shows the glycogen content of erythrocytes in 5 of the patients with GSD type III Studies of the relatives of the patients and of the healthy controls failed to show reproducible values of any magnitude

DISCUSSION

The method of determination of amylo-1 glucosidase activity appears to be reproducible Samples taken from the same subject before and after a meal showed almost the same values (Table 3) Short lasting changes in the amylo-1 6 glucosidase activity of the erythrocytes seem therefore unlikely Larger variations were found when some months elapsed between the different times of sampling from the same person The levels were, however about the same

Our data on amylo 1 6 glucosidase activity of erythrocytes from patients with type III GSD and their parents corresponded well with the mean values (0 102 and 1 01 units/g Hb respectively) reported by Van Hoof (16) Our normal material had a higher mean and a larger range than that reported by Van Hoof in spite of the fact that the same technique was used in the two investigations Whether this is due to hereditary or environmental differences is difficult to say It is more likely due to difference in the branching of the glycogen used for the incubation or in some other factors included in the procedure

The differences between the means of enzyme activity of the patients versus healthy controls and of parents versus healthy controls were relatively larger in this study than reported by Van Hoof The mean activity of 8 parents was only 30 % of that of healthy controls compared to 55 % observed by Van Hoof

From several studies (2 14 15 18) including the present results it appears that probable heterozygotes may be detected by studying the amylo-1 6-glucosidase activity in the blood

corpuscles from relatives of patients with GSD type III In our pooled data on 57 relatives of the patients more than one third had amylo-1 6-glucosidase activity of erythrocytes below the lowest activity observed in the healthy controls Our material is the largest number of relatives in a single investigation and included siblings parents siblings of the parents and paternal and maternal grandparents Huijing *et al* (6) were unable to detect heterozygotes by studying the overall activity of the leucocyte debranching system in 31 relatives of patients with GSD type III All the relatives showed values within the normal range The discrepancy between their results and those of other groups (2 14 18 19) may well be caused by the use of different techniques in the leucocyte studies Huijing *et al* (6) measured the glucose production from phosphorylase limit dextrin while the other authors used incorporation of glucose into glycogen by the reverse reaction of amylo-1 6-glucosidase This latter technique was also used in the erythrocyte studies performed by Van Hoof and in the present investigation According to Huijing *et al* both amylo-1 6 glucosidase and maltotriosyltransferase are required in their test system while the incorporation of glucose into glycogen measures only the amylo-1 6-glucosidase reaction The authors suggested that the overall reaction would not be affected in their test system as long as the transferase activity is still limiting

Van Hoof (15) demonstrated that the glycogen content of erythrocytes of heterozygotes was intermediary between normal and pathological situations although the results varied greatly from one examination to another in the individual cases High glycogen content of erythrocytes was also found in the patients with type III GSD in this investigation In our hands however the determination of glycogen content was not suitable for genetic studies

The biochemical changes in relatives of patients with GSD type III confirm the clinical assumption that most cases of this particular type of GSD are inherited by an autosomal recessive trait In 1966 Brandt & De Luca (1) reported GSD type III in a mother and her 3 children suggesting a dominant type of inheritance Since the parents were first cousins however a recessive type of inheritance was also considered

The existence of subgroups of GSD type III in which the defect is incomplete has been demonstrated by several authors (2 4 8 16) In 1967 Van Hoof & Hers (16) classified GSD type III into 4 subgroups based on biochemical analyses of liver and muscle biopsies Brandt & De Luca (1) suggested that leucocyte studies must also be considered in this subgrouping Although the data concerning the subgroups are still confusing the different degrees and localisations of the enzyme defect in patients with GSD type III make it difficult to exclude the possibility of a dominant type of inheritance in some of the cases

A high incidence of consanguinity has been reported in families with cases of GSD type III indicating a recessive type of inheritance (1 10 12) Consanguinity occurred in 3 of the 4 families with GSD type III examined by us and there was a familial relationship between 2 of them This probably explains the occurrence of 6 definite and 2 probable cases of GSD type III in a relatively small population (about 250 000 *live births*) In a comprehensive study Öckerman (20) was only able to trace a total of 13 definite or highly probable cases of GSD in Sweden This implied an incidence of 1 246 000 live births Only one of his cases was definite and one tentative type III

This particular type of inborn error of metabolism seems to offer special advantages for genetical investigations It should be possible to detect families with probable heterozygote carriers by performing screening studies in isolated areas with known cases of GSD type III Enzymatic studies may also be of value in genetic counselling The overlapping between normal and heterozygote values of amylo-1 6-glucosidase activity must, however be borne in mind

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From several studies (2 14 15 18) including the present results it appears that probable *heterozygotes may be detected* by studying the amylo-1 6 glucosidase activity in the blood

with glycogen storage disease *Clin Chim Acta* 8 807 1963
15 Van Hoof F Amylo-1 6-glucosidase activity and glycogen content of the erythrocytes of normal subjects patients with glycogen storage disease and heterozygotes *Europ J Bioch* 2 271 1967
16 Van Hoof F & Hers H G The subgroups of type III glycogenosis *Europ J Bioch* 2 265 1967
17 Wallis P G Sidbury J B Jr & Harris R C Hepatic phosphorylase defect Studies on peripheral blood *Am J Dis Child* 111 278 1966
18 Williams C & Field J B Studies in glycogen storage disease III Limit dextrinosis A genetic study *J Pediat* 72 214 1968
19 Williams H E Kendig E M & Field J B Leucocyte debranching enzyme in glycogen storage disease *J Clin Invest* 42 656 1963
20 Öckerman, P A Glycogen storage disease in Sweden. *Acta Paediat Scand* Suppl *160* 1965

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Our material demonstrates that in most cases it is possible to diagnose GSD type III without performing liver and muscle biopsies Two of Van Hoof's (15) patients with this disorder however showed an amylo-1 6 glucosidase activity of erythrocytes within or above normal limits If Van Hoof's and our materials are pooled together 27 of a total of 29 cases showed a homozygote level of amylo-1 6 glucosidase activity in erythrocytes with the incubation technique

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SUMMARY

In 20 cases of glycogen storage disease (GSD) from 14 families in Norway genetic studies were performed in 4 families with GSD type III Consanguinity occurred in 3 of the 4 families and familial relationship existed between 2 of them Six cases in the 4 families had clinical findings compatible with a diagnosis of GSD Homozygote values of amylo 1 6 glucosidase activity were found in erythrocytes from 5 studied cases Family histories disclosed that 2 relatives of the patients died with probable GSD

The mean amylo 1 6 glucosidase activity of erythrocytes from 8 parents of the patients was only 30% of that of healthy controls Moreover 20 out of 57 relatives of the patients had low enzyme activity suggesting that they were heterozygotes These relatives included siblings, parents siblings of parents and grandparents

The results are compatible with the assumption that GSD type III is inherited by an autosomal recessive trait

In most cases of GSD type III the diagnosis can easily be made by determining the amylo-1 6 glucosidase activity in erythrocytes from the patients This type seems to offer special advantages for genetic studies

REFERENCES

1 Brandt I K & De Luca V A Type III glycogenosis A family with an unusual tissue distribution of the enzyme lesion *Amer J Med* 40 779 1966
2 Chayoth R Moses S W & Steinitz K Debrancher enzyme activity in blood cells of families with type III glycogen storage disease A method for diagnosis of heterozygotes *Israel J Med Sci* 3 422 1967
3 Cori G T Glycogen structure and enzyme deficiencies in glycogen storage disease *Harvey Lectures Series* 48 p 145 1952 53
4 Hers H G Amylo 1 6 glucosidase activity in tissues of children with glycogen storage disease *Biochem J* 76 69 1960
5 Hers H G Verhue W & Van Hoof F The determination of amylo 1 6 glucosidase *Europ J Bioch* 2 257 1967
6 Huijing F Klein Obbink H J & van Creveld S The activity of the debranching-enzyme system in leucocytes A genetic study of glycogen storage disease type III *Acta genet* (Basel) 18 128 1968
7 Høyer S E & Moe P J Glykogenose *Tidsskr Norske Lægeforen* 87 614 1967
8 Illingworth B A & Brown D H Glycogen storage disease types III IV and VI in W J Whelan & M P Cameron (eds) *Control of glycogen metabolism* Ciba Foundation Symposium J & A Churchill Ltd London 1964 p 336
9 Kluge T Glycogen storage disease A case of generalized glycogenosis type 2 (Pompe's disease) *Acta Path Microbiol Scand* 65 340 1965
10 Levin S Moses S W Chayoth R Jagoda N & Steinitz K Glycogen storage disease in Israel A clinical biochemical and genetic study *Israel J Med Sci* 3 397 1967
11 Moe P J Iron requirements in infancy Longitudinal studies of iron requirements during the first year of life *Acta Paediat Scand* Suppl 150 1963
12 Sidbury J B Jr The genetics of the glycogen storage diseases in A G Steinberg & A G Bearn (eds) *Progress in medical genetics* Grune & Stratton Inc New York 1965 vol IV p 32
13 Skullerud A Myopati ved glykogenoser *Nord Med* 81 379 1969
14 Steinitz K Bodur H & Arman T Amylo-1 6 glucosidase activity in leucocytes from patients

Table 1 *Poliovirus antibody status 3 years after vaccination with trivalent oral vaccine*

	Group of children			
Antibody titre ≥1 10	I (oral vaccine only)		II (inactivated vaccine + oral vaccine)	
Against all three types	12/28[a, b]	(19/28)	18/23[b]	(23/23)
Against type 1	25/28[b]	(24/28)	19/23[b]	(23/23)
Against type 2	31/31	(32/33)	29/30	(30/30)
Against type 3	16/33	(29/33)	26/30	(28/30)
No detectable antibody	1/28	(0/28)	0/23	(0/23)

umerator number of children with antibody Denominator number of children investigated
ue to a technical error five blood samples in group I seven in group II were not tested against poliovirus e 1
umbers in brackets results with sera from the same ldren 1 month following the vaccination in 1966

In the present study the most prominent ding is the lack of demonstrable antibody ainst poliovirus type 3 in 17 of the 33 ildren in group I as compared with only five gatives among the same children 1 month ter the vaccination in 1966

The lack of antibody may be due to an itial response with lower antibody titre gainst the type 3 component of the vaccine r due to a more pronounced fall in antibody tre for type 3 than for types 1 and 2

The geometric mean antibody titre for hildren in group I in 1966 was lower for ype 3 than for types 1 and 2 i e 1 86 1 137 and 1 40 against types 1 2 and 3 espectively In order to compare the decline n antibody titre children with the same titre in 1966 against one virus type were grouped together Starting from equal titre values in 1966 more children seemed to have lost antibody against type 3 than against types 1 and 2 Accordingly there is an indication that both of the mechanisms suggested above may have been operating The number of children investigated is small and the antibody titres were determined in 1966 and 1969 respectively and by somewhat different techniques The results therefore do not permit firm conclusions

However the results certainly indicate that further investigations of the antibody status after oral vaccination is warranted and such investigations become more important in view of the fact that oral vaccine has been used extensively in Norway since the autumn of 1965

SUMMARY

Neutralizing antibodies against poliovirus were investigated in 63 10-year-old children 3 years after oral poliovirus vaccination The antibody status was compared with the results obtained when the same children were examined 1 month after the oral vaccination

A marked change had taken place in the antibody status against poliovirus type 3 among the 33 children who had received only oral vaccine Seventeen of them lacked demonstrable antibody against type 3 after 3 years as compared with only four negatives 1 month after the vaccination The antibody status for these children against poliovirus type 1 and 2 had not undergone any major change Possible mechanisms for this difference are briefly discussed

The 30 remaining children had received inactivated poliovirus vaccine before the oral vaccination and for these children no major changes were observed in the antibody status against the three poliovirus types

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REFERENCES

1 Cesario T C, Jones V Poland J D & Chin T D Y Persistence of neutralizing antibodies four years after immunization with live attenuated vaccine *Amer J Epidem* 90 157 1969
2 Just M & Ritzel G Die Immunität gegen Poliomyelitis 4 bzw 4 Jahre nach oraler Impfung *Schweiz Med Wschr* 96 1710 1966

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POLIOVIRUS ANTIBODY STATUS THREE YEARS AFTER VACCINATION WITH TRIVALENT ORAL VACCINE

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In 1966 the serologic response to oral poliovirus vaccination was investigated in a group of school children in Oslo (7) The antibody status of the same children 3 years after the vaccination is presented in this communication

MATERIAL AND METHODS

In the previous study blood samples were collected from 80 children then aged 7 years before and 1 month after completed vaccination with three doses of trivalent oral poliovirus vaccine According to the manufacturer (Glaxo Laboratories Ltd) one dose of vaccine contained 10 TCID 50 of type 1 poliovirus 10^{5} TCID 50 of type 2 and 10 TCID 50 of type 3

In February 1969 new blood samples were collected from 63 of the 80 children Of these 63 children 33 had received only the oral vaccine (group I) whereas 30 children had also been given inactivated poliovirus vaccine in infancy (group II)

The collection of the blood samples on filter paper discs and the further treatment were performed as described previously (7) Two alterations were adopted for the neutralization tests (*a*) Wild poliovirus strains were replaced by the attenuated Sabin strains No difference in serum titre had been detected in preliminary experiments (*b*) The serum virus mixture was incubated for 3 hours at 37 C and then overnight at 4 C before inoculation into monkey kidney tissue culture tubes This technique is more sensitive than the incubation for 1 hour at 37 C used in the earlier assays

The amount of blood absorbed by the filter discs is sufficient for only one examination by the neutralization test The antibody status in 1969 is therefore compared with the results obtained after the vaccination in 1966

RESULTS

The results of the serological investigations are summarized in Table I where the two groups of children are considered separately The corresponding results obtained in 1966 for the same children, are also presented

A remarkable finding is the antibody status against poliovirus type 3 among the children in group I more than half of them being without demonstrable antibodies (titre < 1 10) whereas most of these children have antibodies against types 1 and 2 Most of the children in group II have antibodies against all three virus types

In group I one child is without demonstrable antibodies against all three virus types, 3 years after vaccination

In the same group one more child possesses antibody against poliovirus type 1 in 1969 as compared with the status 3 years previously probably because of a poliovirus infection in the interval

COMMENTS

Several authors have reported a decline in antibody titre during the years following oral poliovirus vaccination The antibody deficit has been most pronounced for poliovirus type 3 (1–6)

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LIVER FUNCTION STUDIES AND BILIARY TRACT INVESTIGATIONS IN MUCOVISCIDOSIS

JEAN FEIGELSON YVETTE PECAU and JACQUES SAUVEGRAIN

Paris France

ormalities of the liver and biliary tract have ecently been reported in cystic fibrosis of pancreas (2 3 4 5 7 8 18 20 24 25 32 35) Liver cell atrophy fatty infiltra i periportal fibrosis and proliferation of the e ducts have been found Small shrunken llbladders have been described in about 23% cases studied (5 8) including small children and therefore may be congenital in origin In a series of 30 children with cystic fibrosis found that 11 of the patients manifested a ks of abdominal pain which were oc sionally severe The attacks might have re cted the development of pancreatic or in tinal lesions referred abdominal pain from lmonary lesions or hepatic or gallbladder sease Since 5% of such patients develop rrhosis of the liver we studied liver function sts and made radiological investigations of e biliary tract in the hope of earlier detection f liver disease

MATERIAL AND METHODS

Thirty patients with cystic fibrosis 10 males and 20 females aged 18 months to 18 years were studied The diagnosis was established by clinical criteria chest X ray findings, stool analyses and sweat chloride determinations (Table 1) Two children (patients 20 and 30) had overt clinical symptoms of cirrhosis of the liver

The following liver function tests were routinely performed serum glutamic-oxalacetic and glutamic pyruvic transaminase (SGOT & SGPT) (23) thymol turbidity MacLagan test (19) prothrombin test (22) and alkaline phosphatase levels (14) Elimination rate of bromsulphthalein (BSP) was determined by estimation of the clearance of the dye after collection of serial blood specimens between 0 and 15 min after injection (10 17 21)

Radiological investigations of the biliary tract included oral cholecystography and in those cases in which satisfactory opacification of the bile ducts was not obtained intravenous cholangiography with radioselectan

RESULTS

The clinical radiological and biochemical findings are presented in Table 1 *Liver function tests* Thymol turbidity and transaminases (SGOT & SGPT) were normal in all cases except two (nos 22 and 29) who had elevated levels during an attack of infectious hepatitis Serial determinations of prothrombin were usually normal between 70 and 100% but in some instances fell to 40 In patient 20 aged 4 who had cirrhosis and had been operated on for neonatal ileus the prothrombin level fell as low as 28% 2 months before his death Clearances of BSP were significantly abnormal in the two overtly cirrhotic patients BSP half life was 17 min in patient 20 and 15 min in patient 30 BSP half life was slightly more than the normal level (5.5 min) in 5 patients nos 1 10 12 21 26 Seven children (patients 8 12 19 20 24 27 30) had alkaline phosphatase levels over 13 Bodansky

3 Melnick J L Burkhardt M Taber L H & Erckmann P N Developing gap in immunity to poliomyelitis in an urban area *JAMA 209* 1181 1969

4 du Pan R M Bavaud Ch & Koechli B Bilan de l'immunité de la population scolaire de Genève après quatre ans de vaccination orale contre la poliomyélite *XIth Europ Sympos of Poliomyelitis 1966* Masson & Cie Paris 1967 p 173

5 Vonka V Janda Z Simon J Adam E & Stárek M A new type 3 attenuated poliovirus for possible use in oral poliovirus vaccine *Progr Med Virol 9* 204 1967

6 WHO Memorandum Evidence on the safety and efficacy of live poliomyelitis vaccines currently in use with special reference to type 3 poliovirus *Bull WHO 40* 925 1969

7 Örstavik I Flugsrud Liv B & Labelk O Poliovirus antibody status and the response to trivalent oral poliovirus vaccine in Norwegian school children *Acta Pædiat Scand 57* 405 1968

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Table 1 *Comparison of clinical findings radiological and laboratory results*

Case no	Sex	Sweat test Cl mEq/l	Age in y at X ray	Pulm & gen condition	Cyclic abdominal pain	Liver symptoms	BSP [illegible]
Normal gallbladder							
1	♀	107	4	Moderate	+	—	6 5
2	♀	110	7	Severe	−	—	4 5
3	♀	97	2½	Moderately well	−	—	4
4	♀	113	12	Moderately well	−	—	4
5	♀	124	5	Good	−	—	4
6 (†)	♀	132	7	Severe	+	—	3
7	♀	125	9	Moderate	+	—	4
8	♀	150	9	Good	−	—	5
9	o	102	13	Moderately well	+	—	3 5
10	♀	116	4	Good	+	—	6
11	♀	145	10	Good	+	—	3 5
12	♀	105	5	Moderately well	+	Hepatosplenomegaly	7 4
13	♂	86	3	Good	−	—	3 5
14	♀	101	3	Good	−	—	nl in
15	♂	126	12	Moderately well	−	—	7 times
16	♀	95	1½	Good	−	—	
17	♀	137	7	Moderately well	+	—	5
Microgallbladder							
18	♂	121	5	Moderately well	−	—	3 5
19	♀	87	2½	Good (Mec ileus)	−	—	
20 (†)	♂	127	4	Severe (Mec ileus)	−	Cirrhosis (congenital)	17
21	♀	78	12	Moderate	−	—	4
22	♂	117	8	Moderate	+	—	5
23	♀	110	1½	Moderate	−	—	nl in 4 times
24	?	142	3	Good	−	—	4
25	♂	100	5	Good	−	—	5
26	♀	91	10	Moderate	−	—	7
No visualization of the gallbladder							
27	♂	110	2	Moderately well	−	—	nl in 2 times
28	♀	125	9	Moderate	+	Hepatosplenomegaly	3 5
29	♂	139	18	Moderate	−	—	15
30	♂	104	7	Moderate	+	Cirrhosis (Oesoph varices)	

units The higher levels were found in the two cirrhotic patients, and in the patient operated on for intestinal atresia (no 19) A high alkaline phosphatase level was also found in patient 8 who had mild pulmonary symptoms no hepatomegaly and was in good general condition *Radiological investigation* In 17 out of 26 patients the gallbladder appeared to be normal in size as evaluated by comparison with vertebral and rib outline (4 13 28) We considered as normal the gallbladders of 2 patients (8 and 10) which seemed slightly enlarged with slow emptying but presenting a normal duct on the final film In 9 cases gall bladder morphology was definitely abnormal It was small and shrunken from 1 to 1 5 cm long and 0 5 cm wide (Figs 1 2 and 3) The best descriptive term for this finding was felt to be 'microgallbladder' Dye concentration ability homogeneity of the stain and contrast

...n phosphatases ...ky U		Prothrombin lower level in per cent
...level	Higher level	
	9	80
	8 5	55
	13	90
	11 5	90
	1	100
		100
	10	45
	19	100
	7	80
	11	80
	7	85
	16	75
	13	80
	10 5	100
	9	60
	9 5	90
		100
	9 5	45
	19 5	40
	21	28
	3	72
		85
	10	80
	16	65
	10 5	90
	12	85
	17	72
	7 5	90
		76
	47	90

tion after a fatty meal were normal despite their size and morphology This aspect was highly characteristic and specific in the children We did not observe any intermediate stage of doubtful morphology The differentiation between a normal gallbladder and a microgallbladder could definitely be made in all cases Irregularities of the microgallbladder outlines were present in 3 cases and could have been due to the presence of submucosal cysts similar



Fig 1 Microgallbladder patient 20 18 months old

to those described by Esterly & Oppenheimer (8) (Fig 2) Microgallbladders were demonstrable in both the patients with a history of meconium ileus In 4 cases cholecystograms were negative (patients 27 28 29 30) but in two of them the main biliary tract was opacified by contrast medium and appeared normal

DISCUSSION

No direct relationship was found between abdominal pain and radiological abnormalities of the biliary tract Only 3 patients with radiological abnormalities had cyclic abdominal pain One had a microgallbladder with no cyst and two had no visualization of the biliary tract Eight children who suffered from abdominal pain had a normal gallbladder Six children with microgallbladders including those with submucosal cysts did not have any abdominal cyclic pain

There was a good correlation between BSP half life and the clinical hepatic stage BSP test confirmed the diagnosis of cirrhosis in 2 patients In 5 children BSP half life was between 6 and 7 min This might suggest the first stage of an evolution toward cirrhosis and

Table 1 *Comparison of clinical findings radiological and laboratory results*

Case no	Sex	Sweat test Cl mEq/l	Age in y at X ray	Pulm & gen condition	Cyclic abdominal pain	Liver symptoms	BSP bil. ma
Normal gallbladder							
1	♀	107	4	Moderate	+	—	6 5
2	♀	110	7	Severe	–	—	4 5
3	♀	97	2½	Moderately well	–	—	4
4	♀	113	12	Moderately well	–	—	4
5	♀	124	5	Good	–	—	4
6 (†)	♀	132	7	Severe	+	—	3
7	♀	125	9	Moderate	+	—	4
8	♂	150	9	Good	–	—	5
9	♂	102	13	Moderately well	+	—	5 5
10	♀	116	4	Good	+	—	6
11	♀	145	10	Good	+	—	3 5
12	♀	105	5	Moderately well	+	Hepatosplenomegaly	7
13	♂	86	3	Good	–	—	4
14	♀	101	3	Good	–	—	3 5
15	♂	126	12	Moderately well	–	—	nil 2 h
16	♀	95	1½	Good	–	—	
17	♀	137	7	Moderately well	+	—	5
Microgallbladder							
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No visualization of the gallbladder							
27	♂	110	2	Moderately well	–	—	nil 2 m
28	♀	125	9	Moderate	+	Hepatosplenomegaly	3 5
29	♂	139	18	Moderate	–	—	
30	♂	104	7	Moderate	+	Cirrhosis (Oesoph varices)	15

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function The multiple factors which control alkaline phosphatase make difficult the interpretation of their increase We have occasionally found low prothrombin levels This supervision is important from a practical point of view as it can be corrected by vitamin K1 administration

There has been correlation between radiological abnormalities of the biliary tract and liver function tests in only 3 patients patient 20 had meconium ileus microgallbladder cirrhosis and a BSP half life of 17 min patient 30 had a cirrhosis no visualization of the gallbladder and a BSP half life of 15 min in patient 19 who was operated on for a neonatal ileus and had a microgallbladder the BSP could not be performed but the alkaline phosphatase level was high

There was no relationship between gallbladder abnormalities demonstrated by radiological means and the patients age Normal and abnormal gallbladders were found at all ages In patient 7 we performed a second cholecystogram 2 years after the first examination because of the development of recurrent and severe abdominal pain The second cholecystogram was normal as had been the previous one Nonetheless it holds true that there can still be a whole range of degrees of impairment of the gallbladder depending on the amount and the viscosity of the secreted mucus Under certain conditions the output of a stringy mucus may result in an occluded gallbladder at an advanced stage of the disease We did not observe this alteration but we did note that in two of our patients with negative cholecystograms the contrast medium opacified the main biliary tree (28) a fact in favour of an obstruction of the accessory biliary tract The lack of opacification of the gallbladder might also be a consequence of the alteration of the wall (16)

The microgallbladders observed were not radiologically occluded They were functioning properly and capable of concentrating contrast media Histological studies (5 8) have shown their mucosa free of inflammatory changes and a high frequency of multilocular cysts in their submucosa Those seen on X ray pictures display what seems to be an overdevelopment of the same phenomenon We therefore have to concur with Esterly & Oppenheimer (8) who considered the gallbladder to be either intact or affected in utero with no intermediate stage The microgallbladder does not seem to be a consequence but rather an occasional primary congenital feature of cystic fibrosis It can be supposed that this involvement of the gallbladder is a variant of the occasional changes of other organs with a common endodermal origin such as liver intestine and pancreas

The gallbladder is not by itself an essential organ Cholecystograms are none the less worthy of interest in the study of pathophysiology of cystic fibrosis The existence of microgallbladders is another witness of congenital lesions in this disease Survival of patients with mucoviscidosis depends essentially on the state of their lungs Hepatic supervision is however important liver function tests permit us to confirm or to complete clinical impressions and facilitate in some cases the correction of certain deficiencies

SUMMARY

Liver function tests and radiological investigations of the biliary tract were performed in 30 patients with cystic fibrosis of the pancreas

Bromsulphthalein excretion tests (BSP half life) were performed in 27 children They were normal in 20 children 5 patients had moderate changes and 2 patients with cirrhosis had definitely pathological tests Alkaline phosphatase levels were often increased Serial determination of the prothrombin level was usually normal but fell in some instances to 40 of the normal and even as low as 28% in a cirrhotic patient

Radiological investigation of the biliary tract showed marked changes in 13 patients Four patients had no visualization of the gallbladder In 9 cases cholecystograms showed a highly



Fig 2 Microgallbladder with possible multilocular cysts girl 10 years (patient 26)

requires special supervision In patient 6 BSP was performed 1 month before she died and autopsy did not show any hepatic lesion There was a correlation between high alkaline phosphatase level and BSP half life increases in 3 patients the two cirrhotic ones and the patient with a BSP half life of 7 min who had hepato splenomegaly However one child with a constant high alkaline phosphatase level was in good general condition and had normal hepat



Fig 3 Microgallbladder (another form) boy 5 years of age (patient 25)

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characteristic finding a small shrunken microgallbladder with occasionally irregular contours presumably due to submucosal cysts

REFERENCES

1 Alagille D Le complexe prothrombique dans l exploration fonctionnelle de la cellule hépatique *Rev Int Hepat 4* 1 1954

2 Alagille D & Le Tan Vinh Les localisations hepatiques de la mucoviscidose *Gastro Entr 4* 435 1961

3 Andersen D *Cystic fibrosis of the pancreas and its relation to celiac disease* clinical and pathological study *Amer J Dis Child 56* 344 1938

4 Antonelli M Durio A & Marino P La colecistografia orale ed endovenosa in 17 bambini affetti da fibrosi cistica *Atti del XVIII Congresso Nazionale di Radiologia Medica e Medicina Nucleare 4* 293 Padova 1968

5 Bodian M *Fibrocystic disease of the pancreas* Heinemann London 1952

6 Brunnhage F & Enell H Mucoviscidos *Svensk Lakartidn 4* 1335 1961

7 Craig J Haddad M & Shwachman H The *pathological changes in the liver in cystic fibrosis* of the pancreas *Amer J Dis Child 93 357 1957*

8 Esterly J & Oppenheimer E Observation in cystic fibrosis of the pancreas I the gallbladder *Bull John Hopk Hosp 110 247 1962*

9 Farber S Pancreatic function and disease in early life V Pathological changes associated with pancreatic insufficiency in early life *Arch Path 37* 238 1944

10 Fauvert R Benhamou J P & Loverdo A Considerations generales sur la notion de clearance *hepatique Rev Int Hepat 7 429 1958*

11 Gracey M Burke V & Anderson C M Treatment of abdominal pain in cystic fibrosis by oral *administration of n acetyl cysteine Arch Dis Child 44* 404 1969

12 Hennequet A Mucoviscidose *Encycl med chir 6* 4060 1967

13 Jones M Sakai H & Rogerson A Intravenous cholangiography in children with cystic fibrosis of the pancreas *J Pediat 59* 172 1958

14 Klein B Read P A & Babson A A rapid method for quantitative determination of serum alkaline phosphatases *Clin Chem 6* 269 1960

15 Kollberg H Diagnostik och behandling av mukoviskidos *Lakartidningen 29 2929* 1969

16 La ser E *Dynamic factors in roentgen diagnosis* Williams & Wilkins Baltimore 1967

17 Lindquist B & Paulsen L Studies on the elimination rate of bromsulphtalein in infants and children *Acta Paediat Scand 48* 233 1959

18 Le Tan Vinh Cirrhose et mucoviscidose *Presse Med 72* 1934 1964

19 MacLagan N F Thymol turbidity test as indicator of liver dysfunction *Brit J Exp Pathol 25* 234 1944

20 Nezelof C Donnees anatomo pathologiques de la maladie fibrokystique du pancreas *Le Pediatre Par 11 7 1965*

21 Polonovski C & Colin J *Explorations biologiques en pediatrie* Expansion Scientifique Française Paris 1963 p 602

22 Quick A J On relationship between complement and prothrombin *J Immun 29 87 193*

23 Reitman S & Frankel S Colorimetric metho for the determination of serum transamina activity *Amer J Clin Path 28* 1 1957

24 Sanes S Miller D Brason F & Gerst O Fatty changes and cirrhosis of the liver in pa tients with pancreatic lithiasis *Arch Int Med 85* 980 1950

25 di Sant Agnese P & Blanc W A distinctiv type of biliary cirrhosis of the liver associated with cystic fibrosis of the pancreas *Pediatrics 3 387 1956*

26 di Sant Agnese P & Vidaurreta A Cystic fibrosis of the pancreas *J Amer med Ass 172* 135 1960

27 di Sant Agnese P & Talamo R Patho genesis and physiopathology of cystic fibrosis of the pancreas *New Eng J Med 277* 1287 1967

28 Sauvegrain J & Feigelson J La cholecystographie dans la mucoviscidose *Ann Radiol 13* XXX 1970

29 Shwachman H Kulczycki L & Koo Tak Khaw *Studies in cystic fibrosis* A report on 65 patients over 17 years of age *Pediatrics 3* 689 1965

30 *Turla B & Antonelli M* Mucoviscidosi con steatosi epatica massiva *Rivista di Clinica Ped 78 487 1966*

31 *Vignon G Pansu D & Chapuy P* Les osteoses decalcifiantes par malabsorption de l'adult *Le Concours Med 22* 5733 1969

32 Webster R & Williams H Hepatic cirrhosis associated with fibrocystic disease of the pancreas clinical and pathological reports of 5 patients *Arch Dis Child 28* 343 1953

33 Williams T Sherman N & Clatworthy H Pancreatitis as cause of abdominal pain *Pediatrics 6* 1019 1967

34 Wissler H & Zollinger H Congenital cystic fibrosis with bronchiectasis *Helv Paediat Acta I* 3 1945

35 Zuelzer W & Newton W The pathogenesis of fibrocystic disease of the pancreas *Pediatrics 4* 53 1949

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FAMILIAL ATAXIC DIPLEGIA WITH DEFICIENT CELLULAR IMMUNITY

A New Clinical Entity

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In 1966 a girl with vaccinia gangrenosa successfully treated with N methylisatin β thiosemicarbazone (Marboran® Burroughs Wellcome & Co) was reported (8) During recovery at the age of 15 months she was found to have an ataxic diplegia which had not been observed previously At that time it was not known whether the neurological defect was 1) a sequela of the severe disease 2) a toxic side effect of Marboran® or 3) unrelated to the ease and its treatment

Later on a younger brother who had not en vaccinated against variola was found to ve an identical neurological syndrome Both lings also showed evidence of deficient cellar immunity At the age of 4 ³/₄ years the d died of generalized varicella during steroid eatment of a hemolytic anemia The post ortem examination reported elsewhere (1) vealed pathological findings in the central ervous system thought neither to be due to e final acute disease nor to the vaccinia gan renosa or its treatment

The aim of this paper is to complete the pre ious reports (1 8) with a detailed neurological ollow up of the two siblings and with im unological data on the younger boy

CASE HISTORIES

Case 1

G H a girl born January 6 1962 First child of healthy non related parents No relatives with known cerebral palsy or immunological disease Pre peri and neonatal history uneventful Birthweight 3000 g

As early as at the age of 9 months during hospitalization for the abnormal reaction to the variola vaccination the girl was considered late in her psychomotor development At that time she was not able to turn round from prone to supine to creep or to sit unsupported Her fine motor skill was more advanced She could grasp adequately and switch over to the other hand In spite of a good general condition during her first months in the local county hospital she did not learn to sit unsupported until about 12 months of age and even then she swayed and sat very unstably As a result of her severe vaccinia gangrenosa she lay listlessly in her bed during that period

At neurological examination when 17 months of age she was unable to sit without support She showed generalized muscular hypotonia truncal tremor and pronounced difficulties to maintain posture in upright position i e dysequilibrium She also showed a slight intention tremor in her hands and head In addition ankle clonus had been observed on some occasions

After recovering from her vaccinia she started to develop but very slowly At the age of 21 months an ataxic diplegia with spasticity of the feet was very apparent The right foot was more spastic than the left Her intellectual capacity was considered to be at a 12 month level and her speech poor No squint was observed

At 29 months she had learnt to kneel unsupported to stand unsteadily with support and to walk with two-hand support Her arms and hands were much better developed than her legs and feet which functioned at about the 10–11 month level

The last neurological examination was made when the girl was 4 / years of age At that time her neurological syndrome had become even more clear cut She could now walk broad based without support A marked valgus insufficiency of her feet masked the tendency to tip-toe gait Ataxia with pronounced dysequilibrium dominated the picture but

she had persistent ankle clonus and extensor plantar response in addition

Two months later she acquired hemolytic anemia and was treated with cortisone but unfortunately contracted chicken pox at the same time and died of a malignant generalized varicella For a detailed autopsy report, see Berg *et al* (1)

Case 2

J A H a boy born September 7 1964 the younger brother of case 1 No other siblings After a normal pregnancy he was born 10 days before term in a *normal delivery Birthweight 3890 g Neonatal period uneventful*

At the age of 3 months he learned to smile and to fix his gaze Otherwise his motor development was soon found to be retarded He had poor head control and was not able to turn around at the age of 12 months One month later he was sent in for neuropediatric examination He was then at 13 months of age found to function at the low motor level of about 2–3 months His neonatal reflexes had disappeared but he had not yet any protectional reflexes He showed pronounced generalized hypotonia At the same time he had brisk reflexes of the legs with a suspected ankle clonus In addition a *proprioceptive body dysfunction was thought probable but not yet significant*

At follow up examination when 19 months old he functioned at a motor level of 4 months At the age of 2 / years he was able to creep to sit unsupported with an unsteady and bent back and to roll over When put in a standing position he was able to stand with two hand support the knees however were markedly hyperextended and the feet were in a position of maximal valgus insufficiency His mental development was slightly retarded but he was able to produce short sentences Neurologically the main finding was pronounced hypotonia and a dysequilibrium syndrome with truncal tremor but no significant intention tremor In addition the distal spasticity of his legs was confirmed by bilateral ankle clonus and extensor plantar response He had no squint and his fundi were normal

At the age of 4 years he had developed further and was able to walk supported by one hand but his gait was dysharmonic with irregular abnormal steps Protectional reflexes in the legs were completely absent His fine motor skill was at about the 2 / year level and far ahead of the rest of his motor function He obviously had different perceptional defects This was most obvious in regard to his corporeal (body image) and extracorporeal awareness The neurological picture was in all essential respects unchanged at this follow up Mentally he was considered to be low normal or slightly subnormal

The boy's general condition was good He was not particularly prone to infections and he had gone through ordinary chicken pox without complications in 1966 Apart from his neurological abnormalities nothing abnormal was found on general physical examination

NEUROPHYSIOLOGICAL AND METABOLIC SPECIAL EXAMINATIONS

EEG was recorded four times (October 1965 April 1966 October 1966 and March 1969) The first three curves were within normal variations The fourth curve recorded at the age of 4 ¹/ years, showed somewhat slow basic activity with a frequency of 7 c/sec but no paroxysmal abnormalities

EMG findings were normal

Conduction velocity rate Right peroneal nerve 52 left 54 m/sec (normal)

Vibration tests Pronounced spastic responses were obtained from the legs and the flexor muscles of the arms

Metabolic screening on 12 hour night urine The findings in various tube and tape tests were normal as also was the pattern at 2 dimensional amino acid chromatography

IMMUNOLOGICAL INVESTIGATIONS

The ability of the patient to develop contact allergy was tested by means of sensitization attempts with 2 4 dinitro chlorobenzene (DNCB) Twelve control persons (3 children *and 9 adults) were given one sensitizing application* of a solution of 2% DNCB in acetone (w/v) Twenty five μl of this solution (=0 5 mg of DNCB) was applied evenly to an area of 3 cm on the back This area was occluded by an impermeable plastic sheet when the solvent had evaporated This occlusive dressing was kept in place for 48 hours as a rule but had to be taken off after 24 hours in one of the children because of an intolerable burning sensation Simultaneously with the application of the sensitizing DNCB solution, a test of the primary toxicity of DNCB was performed by means of occlusive patch tests (Al test® Imeco Astra Agency Co Stockholm Sweden) In this test the allergen is applied on a piece of filter paper with an area of 0 8 cm These patches were moistened with 10 μl of DNCB in absolute ethanol 0 15% 0 10 % and 0 05% (w/v) respectively The patch tests were left in place for 48 hours Seventy two hours after applica-

tion of the tests the response to the different concentrations of DNCB was evaluated If any erythema occurred on any of the patch test sites the concentration of DNCB causing it was not used in the final testing (this happened in two cases with the 0 15 % solution) In this way it was possible to obtain true positive patch test reactions (homogeneous erythema with palpable induration) 12–25 days after the sensitization in 11 out of the 12 control persons The person not responding to the sensitizing attempt was a woman 65 years old Most probably the failure can be explained by her age as it has been shown that elderly people become sensitized against DNCB less readily than younger people (17)

In our patient the first sensitization attempt failed and was therefore repeated this time with the amount of allergen doubled Nevertheless no contact sensitivity was induced

Intracutaneous injection of an extract of Candida albicans resulted in a non significant delayed reaction The patient had never been vaccinated against tuberculosis and therefore no tuberculin test was performed

Lymph nodes from the groin were excised for histo pathological examination These lymph nodes had not been deliberately stimulated with any antigen They contained a few germinal centres and also plasma cells The number of lymphocytes seemed to be decreased all over the node a fact making it difficult to make a firm statement with regard to the development of the thymus-dependent paracortical areas and giving an impression of reticuloendothelial hyperplasia

The differential counts of white blood cells gave rather low values of the lymphocytes as a rule about 500 mm³ The minimum value recorded was 350 lymphocytes/mm³ and this occurred during an episode with acute pneumonia and otitis bilaterally

Lymphocytes from the peripheral blood of the patient were cultured in and without the presence of phytohemagglutinin (PHA) Lymphocytes were separated from the blood as described by Thierfelder (16) The cells (10^6/ml) were incubated at 37 C in Eagles MEM supplemented with 20% autologous serum 0 12 ml of a PHA M (Difco) stock solution was added to each test tube for 72 hours and the cultures were then fed with tritiated thymidine (^{3}H Tdr 1 μc per culture) for 20 hours The cultures were finally harvested according to a method advised by Ling (10) and the incorporation of ^{3}H Tdr into DNA was measured in a liquid scintillation counter (Packard) Included in the test were also triplet cultures of the patient s unstimulated lymphocytes PHA M stimulated normal adult lymphocytes as positive controls and unstimulated normal adult lymphocytes as negative controls These cultures were incubated fed with tritiated thymidine harvested and counted as is described above for the patient s PHA stimulated cultures The number of disintegrations per minute (c p m) in the unstimulated cultures of the patient (mean 40) did not differ significantly from that of the PHA stimulated cultures (mean 38) Mean c p m for the negative control cultures was 43 and 2000 for the positive ones

The capacity of the patient to produce circulating antibodies was assessed by means of determinations of immunoglobulins and of isohemagglutinins When the patient was 13 months old the value of immunoglobulin G (IgG) was subnormal (300 mg/ml) At 4 ½ years all immunoglobulin values tested (IgA IgE IgG IgM) were normal The isohemagglutinins were found to be low when he was 1 ½ years old (weak agglutination of A_1 erythrocytes with undiluted serum) At 5 years isohemagglutinins occurred even in a dilution of the serum of 1/8 The direct Coombs test was negative

DISCUSSION

The neurological syndrome of the two siblings was one of non progressive ataxic diplegia a truncal tremor dominating the clinical picture In all essential respects the abnormalities were the same in the two cases although more pronounced in the boy The neurological picture

corresponded to the so called dysequilibrium state (7) dominated by proprioceptive and perceptional defects and with no or only minor components of intention tremor and dysmetria

The etiology of ataxic forms of cerebral palsy remains in most cases obscure Prenatal factors are considered to have been at work in the majority according to Ingram (9) prenatal factors are the cause of ataxic diplegia in about 50% In familial cases of cerebral palsy, Gustavsson *et al* (6) found in a recent series that ataxic forms were much more frequent than other syndromes The mode of inheritance in ataxic diplegia was considered to be autosomally recessive autosomally dominant or sexlinked recessive Our two cases are believed to belong to the first category

In addition to their ataxic diplegia our two siblings also showed evidence of deficient cellular immunity To our knowledge a familial syndrome of this type has not been reported previously There are similarities to ataxia telangiectasia (Louis Bar syndrome) However clinically the well known features of that syndrome are clearly different from those in our cases Neither telangiectasia nor the typical cerebellar ataxia was present In addition to qualitative differences the ataxic diplegia of our patients appeared to be non progressive with reservation for the limited number of years of follow up The histopathological picture in our autopsy case also differed from that in ataxia telangiectasia In our girl the cerebellum was of normal size at autopsy while in the Louis Bar syndrom it is atrophied (2 14, 15) In our case there was dysplasia of nerve cells in the cortex of the cerebrum and basal ganglia and heterotopic nerve cells were found in the white matter In the cerebellum there was a patchy loss of Purkinje cells probably dysplasia Non specific degeneration was seen in dorsal root ganglia and in the lateral cortico spinal tract (1) These changes in the central nervous system do not tally with those described in ataxia telangiectasia (14 15)

The deficiency in cellular immunity in the girl was proven by her failure to respond to intracutaneous injection of vaccinia antigen in spite of the severe vaccinia gangrenosa (8)

The boy was judged to have a defect in his cellular immunity from the failure of the attempt at contact sensitization with DNCB and from the lack of response to PHA The negative reaction to the test with candida antigen is compatible with this assumption The system producing humoral antibodies on the other hand seemed to be essentially normal as judged from the normal values of the various immunoglobulins Earlier the boy showed subnormal IgG values but these were by no means comparable to the very low values seen in hypogammaglobulinemic states (5) According to the delineation of the lymphoid system presented by Cooper *et al* (3) and by Parrot *et al* (12) the germinal centers belong to the part of this system involved in antibody production According to this view the occurrence of germinal centers in the lymph nodes of our patient thus fits with the normal immunoglobulin levels in his serum The number of lymphocytes in the peripheral blood was low on several occasions of determination No real alymphopenic episodes were noted, however and it seems as if the possible deficiency in the lymphocyte production would be only moderate

The immunological status of the patient thus showed a combination of a defect in his cellular immunity and on some occasions subnormal lymphocyte content in the peripheral blood but a fair production of immunoglobulins A similar picture is seen in many cases of Louis Bar ataxia telangiectasia (13) Also these patients show impaired development of contact sensitivity and some of them have only minor defects in their immunoglobulin production Further their lymph nodes contain follicular structures and often show a reticulo-endothelial hyperplasia

From a purely immunological point of view the DiGeorge syndrome (4) must also be kept in mind This syndrome is characterized by hypoparathyroidism and congenital absence of the thymus normal levels of immunoglobulins

and of peripheral lymphocytes but with a defect in immunological competence particularly in cellular immunity No investigations regarding the possible absence of the thymus were performed in our second patient However the elder sister with the same clinical picture did have a thymus a fact which certainly lessens the similarity with the immunological status in the DiGeorge syndrome

Pure alymphocytosis (11) is also accompanied by thymic dysplasia and an impaired cellular immunity combined with normal immunoglobulins The number of peripheral lymphocytes varied in the patient with this disease reported by Nezelof (11) and on some occasions it was on the same level as in our patient

It is not possible to differentiate the immunological components of the disease shown by our patient from the three above mentioned syndromes by means of the investigations performed The probability of a hereditary trait and the presence of neurological disturbances make a comparison with ataxia telangiectasia the only hereditary neurological syndrome with immunological deficiency hitherto described most relevant However the neurological pictures of our two cases clearly distinguish their disorder from the Louis Bar syndrome

SUMMARY

Two siblings a girl and a boy with an ataxic diplegia and deficient cellular immunity are reported On the basis of the familial occurrence of this syndrome a hereditary genesis seemed likely The girl died of varicella during steroid treatment for hemolytic anemia Neuropathological examination revealed multiple dysplastic changes supporting a prenatal cause of the neurological syndrome This combined neurological and immunological disorder is thought to be a new clinical entity

ACKNOWLEDGEMENTS

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ADDENDUM

After this paper was prepared Case 2 died at the age of 5 ½ years Three months before his death an acute hemiplegia of the right arm and leg was added to the above described neurological syndrome The hemiplegia improved slowly but there was a gradual deterioration in his general condition due to infections (otitis media bilaterally bronchopneumonia and urinary infection) which did not respond to treatment with ampicillin erythromycin nitrofurantoin or mycostatin During the last 6 weeks of his life he had periodical septic fever He was unconscious in the final week

During the course of the illness the micro-ESR was about 60 mm/ 1 hour The total white cell count was between 3 000 and 8 000 while the mononuclear white cells were between 700 and 1 100 There was no eosinophilia

The preliminary autopsy showed a large abscess in the left cerebral hemisphere otherwise there were no abnormalities visible to the naked eye in the brain the meninges or the spinal medulla A small area of necrosis was found in the trachea multiple bronchopneumonic infiltrations and signs of pyelitis in the kidney The mediastinal lymph nodes were enlarged The thymus and the adrenals were macroscopically small A rich growth of *Klebsiella* was found in the brain abscess and on cultivation also in the blood lungs spleen and kidneys After cultivation a growth of *Torulopsis glabrata* was found in the right lung and the spleen

REFERENCES

1 Berg B Hansson O Nordbring F Sourander P Stenram U & Willen R Fatal varicella generalisata in a child with immunopathy and hereditary neurological syndrome A report with autopsy electron microscopy and virus isolation *Virchows Arch Abt A Path Anat* 346 103 1969

2 Centerwall S A & Centerwall, W R Ataxia telangiectasia A familial degenerative disease leading to mental retardation A case report *Amer J Ment Defic* 71 185 1966

3 Cooper M D Peterson R D A & Good R A Delineation of the thymic and bursal lymphoid systems in the chicken *Nature* 205 143 1965

4 DiGeorge A M Congenital absence of the thymus and its immunologic consequences Concurrence with congenital hypoparathyreoidism in Bergsma & Good (eds) *Immunologic deficiency diseases in man* Birth defect original article series vol IV no 1 p 116 1968

5 Gitlin D Janeway C A Apt L & Craig J M Agammaglobulinemia in Lawrence (ed) *Cellular and humoral aspects of the hypersensitive states* Hoeber Harper New York 1959 p 375

6 Gustavson K H Hagberg B & Sanner G

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BLOOD COAGULATION IN CHILDREN WITH CYANOTIC CONGENITAL HEART DISEASE

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Awareness of the danger of operative or post operative hemorrhagic complications of the surgical correction of congenital heart disease (4) was followed by reports of underlying deficiencies in the mechanisms of hemostasis in patients with congenital heart lesions of the cyanotic variety (8 13) Since these early studies numerous others have been published reporting a very wide variety of coagulation disturbances but there has in many instances been a noticeable lack of agreement between the published results The present paper reports on the coagulation status of a group of patients with cyanotic heart disease and compares the results with those obtained in a control group Since a tendency to thrombosis would appear to be at least as significant clinically as a bleeding tendency in these patients this abnormality in hemostasis is also discussed

MATERIAL AND METHODS

Thirty three patients with cyanotic congenital heart disease were studied Seventeen patients were used as controls The criterion for the selection of the control patients was that they must not have been receiving medication, nor have conditions that could conceivably affect their hemostatic systems For the purpose of evaluating the platelet count 19 additional patients admitted previously to this hospital for evaluation of congenital cyanotic heart disease were included in the patient group and platelet counts were performed on 30 healthy children from the child welfare clinic for inclusion in the control group

The age distribution of the patients with cyanotic heart disease (henceforth referred to as the "patient" group) and the control patients (henceforth referred to as the "control" group) is shown in Fig 1

Finally the records of all patients with uncompensated Fallot's tetralogy admitted to this hospital in the preceding 10 years were reviewed and their mean corpuscular hemoglobin (MCH) compared to that of patients with the same condition and spontaneous cerebrovascular accidents There were 61 patients of which 8 developed signs compatible with this diagnosis

Capillary blood was used for platelet and blood counts and for hemoglobin and hematocrit determinations All other tests were performed on venous blood obtained through a siliconized needle and allowed to drip freely into 5 ml plastic test tubes containing a 0 11 M solution of sodium citrate as anticoagulant The volume of this solution was carefully adjusted to the hematocrit The sample was kept on melting ice until centrifuged at 3 000 r p m and 4 C for 10 min The supernatant plasma was removed with a siliconized pipette distributed in plastic test tubes in 1 ml aliquots and examined immediately or stored at 20 C It was attempted to obtain 15 to 20 ml of blood from each patient but this was frequently not possible because of the size of the veins and the high viscosity In a few patients blood was removed with a plastic syringe through a plastic catheter inserted in the saphenous vein prior to heart catheterization

The following tests were performed on venous blood Prothrombin proconvertin (PP) time according to the method of Owren & Aas (20) expressing the combined activity of factors II VII and X Cephalin (partial thromboplastin) time as described by Egeberg (7) Quick's thromboplastin time using human brain thromboplastin Thrombin time using a commercial thrombin preparation (Hoffman La Roche) diluted so as to give a thrombin time of approxi

Identical syndromes of cerebral palsy in the same family *Acta Paediat Scand* *58* 330 1969
7 Hagberg B Sanner G & Steen M The dys equilibrium syndrome in cerebral palsy *Acta Paediat Scand* To be published
8 Hansson O Johansson S G O & Vahlquist B Vaccinia gangrenosa with normal humoral antibodies A case possibly due to deficient cellular immunity treated with N methylisatin β thiosemicarbazone (Compound 33T57 Marboran) *Acta Paediat Scand* *55* 264 1966
9 Ingram T T S *Paediatric aspects of cerebral palsy* E & E Livingstone Ltd Edinburgh and London 1964
10 Ling N R *Lymphocyte stimulation* North Holland Publishing Company Amsterdam 1968
11 Nezelof C Thymic dysplasia with normal immunoglobulins and immunologic deficiency Pure alymphocytosis *in* D Bergsma & R A Good (eds) *Immunologic deficiency diseases in man* Birth Defect Original Article Series vol IV no 1 p 104 1968
12 Parrot D M V de Sousa M A B & East J Thymus-dependent areas in the lymphoid organs of neonatally thymectomized mice *J Exp Med* *123* 191 1966
13 Peterson R D A Cooper M D & Good R A Lymphoid tissue abnormalities associated with ataxia telangiectasia *Amer J Med* *41* 342, 1966
14 Sourander P Bonnevier J O & Olsson Y A case of ataxia telangiectasia with lesions in the spinal cord *Acta Neurol Scand* *42* 354 1966
15 Terplan K L & Krauss R F Histopathologic brain changes in association with ataxia telangiectasia *Neurology* *19* 446 1969
16 Thierfelder S A A method for the isolation of human lymphocytes *Vox Sanguinis* *9* 447 1964
17 Waldorf D S Wilkens R F & Decker J L Impaired delayed hypersensitivity in an aging population *JAMA* *203* 111 1968

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Key words Ataxia diplegia dysequilibrium cellular immunity delayed hypersensitivity 2 4 dinitrochlorobenzene phytohemagglutinin

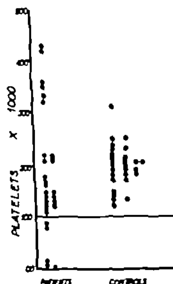


Fig 3 Platelet counts in patients and controls

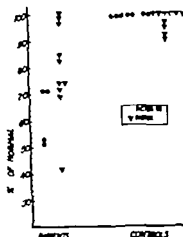


Fig 5 Factor II and VII levels in 10 patients and 10 controls

was employed It was soon evident that patients with high hematocrit could have virtually normal bleeding times despite considerable thrombocytopenia It was assumed that bleeding times which under other circumstances would have been prolonged were being affected by the high viscosity and the test was therefore not performed by routine on these highly polycythemic patients

Platelet count Nineteen of 47 patients and 5 of 35 controls had counts below 150 000 This difference is statistically significant ($p < 0.005$) Furthermore nine of the 47 patients had counts below 100 000 (Fig 3)

Fibrinogen Plasma fibrinogen levels were within (or in isolated instances above) the normal range of 150 to 350 mg/100 ml in 31 of 32 patients and in all of the 16 controls One patient had a level of 70 mg/100 ml If the results were converted to whole blood fibrinogen on the basis of the hematocrit levels 13 of 32 patients and none of the 16 controls had lower than normal values ($p < 0.005$) (Fig 4)

Other coagulation factors It was originally intended to measure individual coagulation factors in all patients with a prolonged cephalin time and/or clinical signs of a hemorrhagic disorder Only 1 patient had an abnormal cephalin time Nevertheless because of the frequency with which a prolonged prothrombin

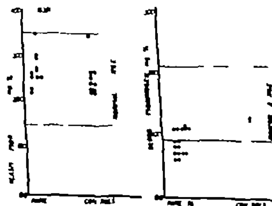


Fig 4 Plasma and whole blood fibrinogen levels in patients and controls

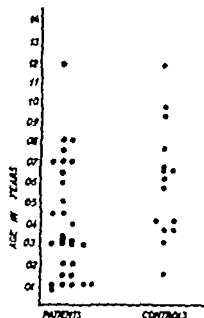


Fig 1 Age distribution of patients and controls

mately 20 sec with normal plasma Fibrinogen concentration determined spectrophotometrically by the method of Jacobsson (16) modified by Blombäck & Blombäck (5) and Godal (11) All the coagulation tests with the exception of the thrombin time were performed in duplicate using 0 1 ml of plasma *Thrombin time was performed in a single test using* 0 2 ml of plasma Only new non siliconized test tubes of a constant diameter were employed

In addition to the tests mentioned above bleeding time (Ivy s method) tourniquet test euglobulin clot lysis time and determination of isolated factors were performed on a number of patients The method for factor II assay was the RVV cephalin method (14) and for factor VII a one stage assay using congenital factor VII-deficient substrate plasma

RESULTS

Cephalin time One patient had a significantly prolonged cephalin time Factors VIII and V were measured in this patient and were 100% and 87% respectively One patient had a slightly prolonged cephalin time and the remaining patients had normal values as did the controls (Fig 2)

Combined factor II VII and X activity In 21 of 30 patients as opposed to 1 of 17 controls the PP method showed values below the lower limit of normal ($p < 0.0001$) (Fig 2)

Quick s thromboplastin time In 2 of 32 patients and in 1 of 15 controls times moderately in excess of the upper limit of normal were found (Fig 2)

Thrombin time The thrombin dilutions employed gave thrombin times with normal plasma which ranged between 17 and 24 sec The results are expressed as the number of seconds above (+) or below (−) the thrombin time which the particular thrombin dilution employed had given with normal plasma No difference could be shown in the thrombin times of 26 patients compared with those of 17 controls (Fig 2)

Bleeding time Ivy s technique using two 1 cm long superficial incisions instead of punctures and a b p cuff pressure of 40 mm H

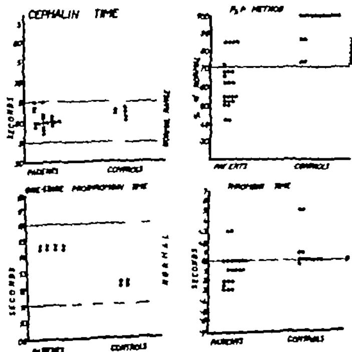
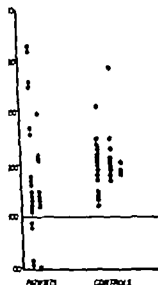


Fig 2 Cephalin time combined II VII and X (PP) value Quick thromboplastin (one stage prothrombin) time and thrombin time in patients and controls



r 3 Platelet counts in patients and controls

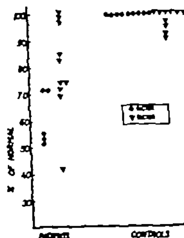


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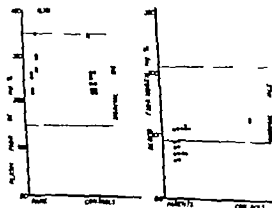


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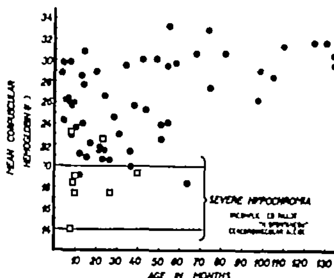


Fig 6 Mean corpuscular hemoglobin of patients with and without spontaneous cerebrovascular accidents in relation to age

proconvertin time was observed factors II and VII were determined in 10 patients with a decreased PP value and in 10 children chosen at random from the control group A statistically significant decrease could be shown for both factors ($p<0\ 001$) the decrease being more marked for factor VII (Fig 5)

Other determinations Euglobulin clot lysis time was determined in 4 patients and 5 controls The values for the patients were 180 90 225 and 270 min and for the controls 85 85 85 120 and 120 min All these results are within the normal range

Analysis of case material Eight of the 61 patients admitted for Fallot s tetralogy had developed signs compatible with the diagnosis of spontaneous cerebrovascular accident Six of these 8 patients had a MCH below 20 whereas only 3 of the 32 patients in the same agegroup as the former but without this complication had such a low value (Fig 6)

DISCUSSION

The results of this study show a statistically significant difference in the concentration of factors II VII and X using the PP method in the concentration of fibrinogen when expressed as whole blood fibrinogen, and in the incidence of thrombocytopenia in children with cyanotic heart disease as compared to a group of children without heart disease No difference could be shown in the cephalin time Quick thromboplastin time or plasma fibrinogen

Coagulation factors Reduction of a specific coagulation factor has been reported either as an isolated or as a combined deficiency for virtually all factors but the variable and partly conflicting nature of the reports and the usually low incidence of the deficiencies described, coupled to a lack of controls (6) has made their interpretation difficult Most studies have shown some lengthening of the Quick thromboplastin time (9 10 13 15) though not to the extent originally described (8) before the need for careful adjustment of anticoagulant to the hematocrit had been demonstrated (9)

A reduction of any one of the factors involved in the cephalin time to a level of 30%-50 will prolong it (12 22) On the basis of this test therefore it can be concluded that in the present patients there was no clinically significant reduction of the plasmatic factors involved in the intrinsic system though minor reductions can not be excluded A reduction in the combined factor II VII and X level as measured by the PP method was established in the patient group Determination of the individual factors in a group of patients and a group of controls showed that the difference is more pronounced for factor VII though it is also present for factor II Factor V levels do not influence the PP method and factor X was not measured but these factors are com

a to both systems and a clinically significant ection of any one of them should have wn up as a prolonged cephalin and/or ark thromboplastin time

Liver damage has been demonstrated in dts with hypoxemia of pulmonary origin () and could underlie the clinically insig icant reductions of factors II and VII found this study

With one exception no decrease in plasma rinogen was found in 32 patients Conversion whole blood fibrinogen values showed a re ction in almost 50 of the patients The nificance of a reduced whole blood fibri gen in the face of a normal plasma concen tion is an open question but it has been nsidered to be of importance (13) presum ly for the mechanical qualities of the clot

Platelets Thrombocytopenia has been a fre ent finding in patients with cyanotic heart sease (10 13 21 27 28) though its fre ency and its relevance to the heart disorder ve been questioned (1 2 3 23) In this udy the platelet count was below 100 000 in 9 5 of the patients A correlation between e platelet count and the arterial oxygen sa ration in cyanotic heart disease has been re orted (17) and thrombocytopenia has also een described in adults with lung insufficiency 18)

Therapeutic considerations

Serious operative or postoperative hemorrhage n patients with congenital cyanotic heart isease operated upon without the use of extracorporeal circulation appears to be re lated to the age at operation its frequency in creasing with age (13) The present policy of early palliative or corrective surgery would thus appear to have largely removed this ha zard Extracorporeal circulation does of course introduce other problems of hemostasis not evaluated here (10) Postoperative thrombosis at the site of an anastomosis or elsewhere is a more likely consequence of the altered hemostasis than hemorrhage and thus measures aimed at its prevention would be welcome Anticoagulants have been advised against (13) and are difficult to use in these polycythemic children Taussig has emphasized the import ance of adequate hydration (26)

Though high viscosity coupled to post operative changes in coagulation and some times inadequate hydration probably plays a part in postoperative thrombosis no correlation with a high hematocrit appears to exist for spontaneous cerebrovascular accidents (19) Thus cerebral thrombosis is commonest in the first year of life an age in which very high hematocrit levels are uncommon Instead there appears to be a correlation with a low MCH (19) and the findings in this study support this hypothesis (Fig 6) The therapeutic implica tion is straightforward The same age associated factors which frequently lead to iron deficiency in small children will also be acting in those with cyanotic heart disease Their hematological status should be closely watched in infancy and early childhood and supplementary iron given if the MCH starts to decrease

SUMMARY

A comparison of the coagulation status of 33 children with cyanotic congenital heart disease to that of a control group is presented The patient group showed a statistically significant decrease in the concentration of factors II and VII and in the concentration of fibrinogen when expressed as whole blood fibrinogen and a statistically significant increase in the inci dence of thrombocytopenia No difference could be shown in the cephalin (partial throm boplastin) time thrombin time or plasma fibrinogen concentration Except for the thrombocytopenia none of the deficiencies found in this study seem important enough to give rise to a hemorrhagic syndrome or to be the cause of increased operative blood loss Evidence in the literature indicates that the risk of increased operative blood loss in cyanotic congenital heart disease is greater when the pa tient is an older child or adult Present policies by which severely hypoxemic children with

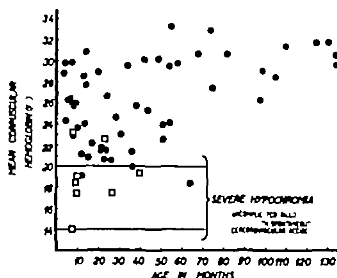


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DISCUSSION

The results of this study show a statistically significant difference in the concentration of factors II VII and X using the PP method in the concentration of fibrinogen when expressed as whole blood fibrinogen and in the incidence of thrombocytopenia in children with cyanotic heart disease as compared to a group of children without heart disease No difference could be shown in the cephalin time Quick thromboplastin time or plasma fibrinogen

Coagulation factors Reduction of a specific coagulation factor has been reported either as an isolated or as a combined deficiency for virtually all factors, but the variable and partly conflicting nature of the reports and the usually low incidence of the deficiencies described coupled to a lack of controls (6) has made their interpretation difficult Most studies have shown some lengthening of the Quick thromboplastin time (9 10 13 15) though not to the extent originally described (8) before the need for careful adjustment of anticoagulant to the hematocrit had been demonstrated (9)

A reduction of any one of the factors in volved in the cephalin time to a level of 30% 50% will prolong it (12 22) On the basis (this test therefore it can be concluded that i the present patients there was no clinicall significant reduction of the plasmatic factor involved in the intrinsic system though minc reductions can not be excluded A reductio in the combined factor II VII and X level a measured by the PP method was establishe in the patient group Determination of the in dividual factors in a group of patients and group of controls showed that the differenc is more pronounced for factor VII, though i is also present for factor II Factor V level do not influence the PP method and factor X was not measured but these factors are com

a to both systems and a clinically significant uction of any one of them should have own up as a prolonged cephalin and/or rk thromboplastin time

Liver damage has been demonstrated in ilts with hypoxemia of pulmonary origin I) and could underlie the clinically insig icant reductions of factors II and VII found this study

With one exception no decrease in plasma rinogen was found in 32 patients Conversion whole blood fibrinogen values showed a re ction in almost 50% of the patients The nificance of a reduced whole blood fibri een in the face of a normal plasma concen tion is an open question but it has been nsidered to be of importance (13) presum ly for the mechanical qualities of the clot

Platelets Thrombocytopenia has been a fre ent finding in patients with cyanotic heart sease (10 13 21 27 28) though its fre ency and its relevance to the heart disorder ve been questioned (1 2 3 23) In this idy the platelet count was below 100 000 in 35% of the patients A correlation between e platelet count and the arterial oxygen sa ration in cyanotic heart disease has been re rted (17) and thrombocytopenia has also en described in adults with lung insufficiency (8)

herapeutic considerations

cious operative or postoperative hemorrhage patients with congenital cyanotic heart sease operated upon without the use of xtracorporeal circulation appears to be re ted to the age at operation its frequency in reasing with age (13) The present policy of arly palliative or corrective surgery would hus appear to have largely removed this ha ard Extracorporeal circulation does of course ntroduce other problems of hemostasis not valuated here (10) Postoperative thrombosis at the site of an anastomosis or elsewhere is a more likely consequence of the altered hemostasis than hemorrhage and thus measures aimed at its prevention would be welcome

Anticoagulants have been advised against (13) and are difficult to use in these polycythemic children Taussig has emphasized the import ance of adequate hydration (26)

Though high viscosity coupled to post operative changes in coagulation and some times inadequate hydration probably plays a part in postoperative thrombosis no correlation with a high hematocrit appears to exist for spontaneous cerebrovascular accidents (19) Thus cerebral thrombosis is commonest in the first year of life an age in which very high hematocrit levels are uncommon Instead there appears to be a correlation with a low MCH (19) and the findings in this study support this hypothesis (Fig 6) The therapeutic implica tion is straightforward The same age associated factors which frequently lead to iron deficiency in small children will also be acting in those with cyanotic heart disease Their hematological status should be closely watched in infancy and early childhood and supplementary iron given if the MCH starts to decrease

SUMMARY

A comparison of the coagulation status of 33 children with cyanotic congenital heart disease to that of a control group is presented The patient group showed a statistically significant decrease in the concentration of factors II and VII and in the concentration of fibrinogen when expressed as whole blood fibrinogen and a statistically significant increase in the inci dence of thrombocytopenia No difference could be shown in the cephalin (partial throm boplastin) time thrombin time or plasma fibrinogen concentration Except for the thrombocytopenia none of the deficiencies found in this study seem important enough to give rise to a hemorrhagic syndrome or to be the cause of increased operative blood loss Evidence in the literature indicates that the risk of increased operative blood loss in cyanotic congenital heart disease is greater when the pa tient is an older child or adult Present policies by which severely hypoxemic children with

congenital heart disease are submitted to palliative or corrective procedures at an early age should minimize the danger of hemorrhage Nevertheless a careful investigation of the coagulation status of all patients with cyanotic congenital heart disease who are to undergo surgical procedures is indicated Thrombosis, especially cerebral thrombosis, is a more important complication than hemorrhage in the pediatric age group Evidence is presented in support of the view that iron deficiency predisposes to this complication possibly by increasing tissue hypoxia

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REFERENCES

1 Alagille D Heim de Balsac R Guery J Passelecq J Blondeau P & Dubost Ch Les thrombopathies associees aux cardiopathies congenitales *Rev Franc Etud Clin Biol 3* 332 1958
2 Alagille D & Goffaux J Le temps de saignement au cours des cardiopathies congenitales *Nouv Rev Franc Hemat 3* 327 1963
3 — Les troubles preoperatoires de l hémostase dans les cardiopathies congénitales *Nouv Rev Franc Hemat 5* 23 1965
4 Bahnson H T & Ziegler R F A consideration of the causes of death following operation for congenital heart disease of the cyanotic type *Surg Gynec Obstet 90* 60 1950
5 Blomback B & Blomback M Purification of human and bovine fibrinogen *Arkiv for Kemi* (Stockholm) *10* 415 1956
6 Burrow L Watson D G & Bell W N Blood coagulation studies in children with congenital heart disease *Amer Heart J 70* 747 1965
7 Egeberg O Assay of antihemophilic A B and C factors by one stage cephalin systems *Scand J Clin Lab Invest 13* 140 1961
8 Favre Gilly J Bret J & Borel Milhet J Un trouble inattendu de la coagulation dans la maladie bleu l hypoprothrombinemie *Sang 22* 278 1951
9 Favre Gilly J Les difficultes d estimation du taux de la prothrombine chez les polyglobuliques *Sang 26* 788 1955
10 Gans H & Krivit W Problems in hemostasis during and after open heart surgery VI Over all changes in blood coagulation mechanisms *JAMA 179* 145 1962
11 Godal H C Simple syneresis procedure for fibrinogen assay *Scand J Clin Lab Invest 13* 530 1961
12 Gonyea L M Hjort P & Owren P A Study of relationship of concentrations of prothrombin proconvertin and proaccelerin to 3 methods for measuring prothrombin time *J Lab Clin Med 48* 624 1956
13 Hartmann R C A hemorrhagic disorder occurring in patients with cyanotic congenital heart disease *Bull Johns Hopkins Hosp 91* 49 1952
14 Hjort P Rapaport S I & Owren P A A simple specific one stage prothrombin assay using Russell s viper venom in cephalin suspension *J Lab Clin Med 46* 89 1955
15 Jackson D P Hemorrhagic diathesis in patients with cyanotic congenital heart disease *Ann NY Acad Sci 115* 235 1964
16 Jacobsson K Studies on determination of fibrinogen in human blood plasma *Scand J Clin Lab Invest 7* Suppl *14* 1955
17 Kornhuber B & Gutheil H Gerinnungsstorungen bei Kindern mit angeborenen Herzfehlern *Z Kreislaufforsch 54* 738 1965
18 Kummer H Gurtner H P & Bucher U Thrombocytopenie bei chronischen Cor pulmonale und cyanotischen Herzvitien *Schweiz Med Wschr 94* 1334 1964
19 Martelle R R & Linde L M Cerebrovascular accidents with tetralogy of Fallot *Amer J Dis Child 101* 206 1961
20 Owren P A & Aas K The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin *Scand J Clin Lab Invest 3* 201 1951
21 Paul M H Currimbhoy Z Miller R A & Schulman I Thrombocytopenia in cyanotic congenital heart disease *Amer J Dis Child 102* 597 1961
22 Proctor R R & Rapaport S I The partial thromboplastin time with kaolin *Amer J Clin Path 36* 212 1961
23 Raby C Les troubles de l hemostase dans les cardiopathies congénitales leur retentissement sur les pertes sanguines post opératoires *Hemostase 2* 279 1962
24 Refsum H E Arterial hypoxaemia serum activities of GO T GP T and LDH and centrilobular liver cell necrosis in pulmonary insufficiency *Clin Sci 25* 369 1963
25 Rudolph A M Nadas A S & Borges W H Hematologic adjustments to cyanotic congenital heart disease *Pediatrics 11* 454 1953
26 Taussig H B *Congenital malformations of the heart* 2nd ed Commonwealth Fund New York 1960

27 van Creveld S Hemorrhagic diathesis in congenital heart disease Influence of operation under hypothermia and of whole blood transfusions *Ann Paediat 190* 342 1958
28 Verel D Mazurkie S J Blackburn E K Emery J L Varadi S & Wolman L Thrombocytopenia in congenital heart disease *Brit Heart J 24* 92 1962

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REFERENCES

1 Alagille D Heim de Balsac R Guery J Pusselecq J Blondeau P & Dubost Ch Les thrombopathies associees aux cardiopathies congenitales Rev Franc Etud Clin Biol 3 332 1958
2 Alagille D & Goffaux J Le temps de saignement au cours des cardiopathies congénitales Nouv Rev Franc Hemat 3 327 1963
3 — Les troubles préoperatoires de l'hemostase dans les cardiopathies congénitales Nouv Rev Franc Hemat 5 23 1965
4 Bahnson H T & Ziegler R F A consideration of the causes of death following operation for congenital heart disease of the cyanotic type Surg Gynec Obstet 90 60 1950
5 Blombäck B & Blombäck M Purification of human and bovine fibrinogen Arkiv for Kemi (Stockholm) 10 415 1956
6 Burrow L Watson D G & Bell W N Blood coagulation studies in children with congenital heart disease Amer Heart J 70 747 1965
7 Egeberg O Assay of antihemophilic A B and C factors by one stage cephalin systems Scand J Clin Lab Invest 13 140 1961
8 Favre Gilly J Bret J & Borel Milhet J Un trouble inattendu de la coagulation dans la maladie bleu l hypoprothrombinémie Sang 22 278 1951
9 Favre Gilly J Les difficultés d estimation du taux de la prothrombine chez les polyglobuliques Sang 26 788 1955
10 Gans H & Krivit W Problems in hemostasis during and after open heart surgery VI Over all changes in blood coagulation mechanisms JAMA 179 145 1962
11 Godal H C Simple syneresis procedure for fibrinogen assay Scand J Clin Lab Invest 13 530 1961
12 Gonyea L M Hjort P & Owren P A Study of relationship of concentrations of prothrombin, proconvertin and proaccelerin to 3 methods for measuring prothrombin time J Lab Clin Med 48 624 1956
13 Hartmann R C A hemorrhagic disorder occurring in patients with cyanotic congenital heart disease Bull Johns Hopkins Hosp 91 49 1952
14 Hjort P Rapaport S I & Owren P A A simple specific one stage prothrombin assay using Russell s viper venom in cephalin suspension J Lab Clin Med 46 89 1955
15 Jackson D P Hemorrhagic diathesis in patients with cyanotic congenital heart disease Ann NY Acad Sci 115 235 1964
16 Jacobsson K Studies on determination of fibrinogen in human blood plasma Scand J Clin Lab Invest 7 Suppl 14 1955
17 Kornhuber B & Gutheil H Gerinnungsstörungen bei Kindern mit angeborenen Herzfehlern Z Kreislaufforsch 54 738 1965
18 Kummer H Gurtner H P & Bucher U Thrombocytopenie bei chronischen Cor pulmonale und cyanotischen Herzvitien Schweiz Med Wschr 94 1334 1964
19 Martelle R R & Linde L M Cerebrovascular accidents with tetralogy of Fallot Amer J Dis Child 101 206 1961
20 Owren P A & Aas K The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin Scand J Clin Lab Invest 3 201 1951
21 Paul M H Currimbhoy Z Miller R A & Schulman I Thrombocytopenia in cyanotic congenital heart disease Amer J Dis Child 102 597 1961
22 Proctor R R & Rapaport S I The partial thromboplastin time with kaolin Amer J Clin Path 36 212 1961
23 Raby C Les troubles de l hemostase dans les cardiopathies congénitales leur retentissement sur les pertes sanguines post operatoires Hemostase 2 279 1962
24 Refsum H E Arterial hypoxaemia serum activities of GO-T OP T and LDH and centrilobular liver cell necrosis in pulmonary insufficiency Clin Sci 25 369 1963
25 Rudolph A M Nadas A S & Borges W H Hematologic adjustments to cyanotic congenital heart disease Pediatrics 11 454 1953
26 Taussig H B Congenital malformations of the heart 2nd ed Commonwealth Fund New York 1960

Table 1 *Clinical data on 8 patients with solitary mastocytoma*

Case	Site of lesion	Age of onset	Darier's sign	Blistering Vesiculation	General symptoms	Co-existent disease	Family history	Signs of systemic involvement
1	Left thigh	Birth	+	+	Flush	–	Urticaria	–
2	Trunk	Two weeks	+	–	Flush	–	Bronchial asthma Allergic rhinitis	–
3	Left forearm	One week	+	–	–	Obstructive bronchitis	Bronchial asthma	–
4	Left forearm	Birth	+	–	Respiratory wheezing	Recurrent obstructive bronchitis	Bronchial asthma	–
5	Left forearm	Nine months	+	+	–	–	–	–(?)
6	Right forearm	Eight months	+	–	–	Neonatal hypoglycemia Convulsive disorder	–	–
7	Sole of right foot	Birth	(–)	+	–	–	–	–
8	Left forearm	Two months	+	–	–	–	?	–

erythema which lasted for 10–15 min The child's general condition was good

Neither the liver nor the spleen were enlarged The left thigh showed a 10 by 22 mm sharply outlined slightly raised plaque (Fig 1) Darier's sign was positive (urtication of the tumour after mechanical irritation) Vesicles formed from time to time on the surface of the skin lesion but no bullae appeared during the child's stay in hospital at 5 months of age and stroking of the lesion no longer produced generalized erythema

The blood picture was normal There was 0 2 basophils and 0 5 eosinophils per 100 white blood cells (1 000 cells counted) The number of bone marrow cells stained metachromatically by toluidine blue was not increased

Histologically a very dense subepidermal aggregation of mast cells was seen The cells had vesicular pale nuclei and fairly scanty cytoplasm which stained strongly with toluidine blue

Case 4

A boy aged 10 months His grandmother had bronchial asthma The boy had recurrent obstructive bronchitis since six months of age Physical exertion caused wheezing

Since birth he had had an 11 by 20 mm light brown and somewhat elevated and infiltrated tumour on his left forearm Darier's sign was positive and the erythema involved a considerable portion of the forearm (Fig 2) On one occasion a typical attack of bronchial asthma without concomitant general flushing was provoked by gentle scratching of the tumour The attack was curtailed by epinephrine and prednisolone

The liver and spleen were not palpably enlarged The blood picture was normal Only a few metachromatic cells were found in the toluidine blue stained bone marrow smear

Histologically the upper part of the dermis contained numerous groups of regular cells with light oval nuclei and rather abundant cytoplasm The cells were arranged in columns and around blood vessels as well as in scattered groups between collagenous fibres in the deep layers of the dermis The cytoplasmic granules were demonstrated by staining with toluidine blue and Giemsa stain

After excision of the tumour the patient continued to have attacks of asthmatic bronchitis The operation wound healed with keloid formation

Fig 3 shows the histological appearance in case no 2

RESULTS

The results of the laboratory studies are summarized in Table 2 The 24 hour urinary excretion of histamine was determined in five of the children it was normal in four but substantially increased in one This patient had generalized flushes though not during the period of collection of the urine The histamine values found in this patient are given in Table

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SOLITARY MASTOCYTOMA

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Mastocytosis is a term used to denote a pathological infiltration of mast cells in the skin or other tissues It may be divided into a *cutaneous* and a *systemic* form Three types of the cutaneous form may be recognized with regard to the extent of the disease *solitary mastocytoma disseminated* and *diffuse* mastocytosis (34) The disseminated type of cutaneous mastocytosis corresponds to the form of urticaria pigmentosa originally described in 1869 (27)

In systemic mastocytosis infiltrations of mast cells are present in such extra-cutaneous sites as bone and bone marrow liver spleen lymph nodes The condition may be fatal even in childhood Much of the interest in mastocytosis is due to the fact that mast cells contain and elaborate substances responsible for many of the manifestations of the disease (21)

Solitary mastocytoma in man was first described in 1934 (17) Mastocytoma is a common tumour in dogs (4) and also occurs in other animals (30) Some 75 cases have so far been recorded in man (6 7 9 10 14 19 20)

This paper reports eight cases of solitary mastocytoma seen within a three year period at the department of paediatrics in Malmö General Hospital

MATERIAL

The series consisted of eight children aged 5–18 months with solitary mastocytoma Judging from skeletal X ray and haematological and bone marrow studies the disease was not systemic Six of the children were seen by the author A search of the records of various clinical departments of the hospital revealed two additional cases of solitary mastocytom and six cases of disseminated urticaria pigmentosa such young children The present material was co fined to the patients with isolated lesions this ty of the disease having received less attention in t literature Clinical data on the eight cases are pr sented in Table 1

METHODS

The excretion of histamine in the urine was estimate in five cases In four the amine was measured b bio assay on isolated guinea pig ileum (37) In on case (no 1) both the histamine and the methylhista mine were estimated and then by a spectrophot metric method (16)

As a rule coagulation studies included coagulatio time and recalcification time of citrated plasma bleed ing time (Duke & Ivy) thrombocytes one stage pr thrombin time with human brain thromboplasti (Quick) P & P (31) factor V (38) fibrinogen d te mination and estimation of fibrinolysis on fibr plates and euglobulin clot lysis time (28) and examin ing of circulating anticoagulant (the effect of the pa tient's plasma on the coagulation time of norma blood and on the recalcification time and prothromb time of normal plasma thrombin titration (29)) I one patient (no 5) the local fibrinolysis in the are of the tumour was tested with a modification c Todd's fibrin slide method (32)

CASE REPORTS

Two cases are described in some detail (no 1 an no 4)

Case 1

A girl aged 5 months Her mother had urticaria The child had had a skin lesion on her left thig since birth At 2 ½ months a blister had appeared o the surface of the lesion The blister had persiste for 14 days and then dried up without rupturing A 4 months rubbing of the tumour induced generalize

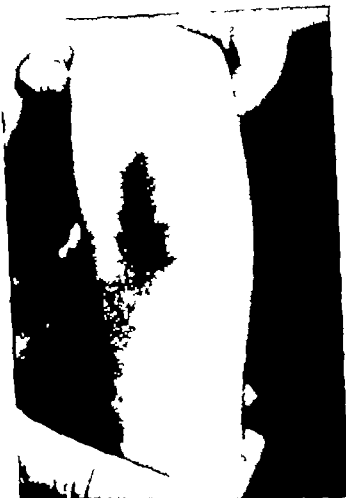


Fig. 2. Solitary mastocytoma on left forearm. Positive Darier's sign. Erythema covers major part of forearm.

s minated form can hardly pass unnoticed and practically all cases of the solitary variant with its fairly conspicuous symptoms must be detected though the lesions may sometimes be mistaken for a common nevus or some other skin disease. A positive Darier's sign was the clue to the diagnosis in all 8 cases. But this sign is not conclusive since a few other cases with a suspected Darier's sign were observed where biopsy failed to confirm the diagnosis. Yet, it must be borne in mind that even histological examination may sometimes miss mast cells undergoing degranulation.

The tumour appeared as a macule in one case, as a papule in one, as a small nodule in one, and in the remaining 5 as a more or less raised plaque. The lesion was noticed at or soon after birth in six of the infants, but not until 8 and 9 months after birth in the remaining two.

In these respects the lesions resembled those on record. Solitary mastocytoma is apparently more often situated on the limbs, especially on the forearm and around the wrist, than on the trunk (9, 34). In one published case the tumour was situated in the sole of the foot (1) as in case 7. Mastocytoma is, as a rule, observed at birth or during the first few weeks or months of life, but it may occur later, even in adult age (19).

No cases of mastocytosis were known among any of the other members of the families. The material thus lent no support to the opinion that mastocytosis is a genetic disease (35). On



Fig 1 Solitary mastocytoma Sharply de marcated and slightly raised reddish pla que on left thigh

3 There may have been a loss of urine during the sampling as the volumes were small A 24 hour sample was collected first The next day the urine was collected in two portions one 8 hour sample and one 16 hour sample The 8 hour sample was collected during the day when the patient was given aspirin and when the tumour was repeatedly stroked

DISCUSSION

Eight cases of solitary mastocytoma have oc cured among children born in the city of Malmo in 1966–1968 which means an incidence of 0 7 per thousand In addition six cases of dis seminated urticaria pigmentosa have been dis covered among the children born in these years Both forms of mastocytosis had invariably ap peared before the children were one year of age The total incidence was 1 3 per thousand

Thus in the present study solitary mastocy toma does not seem to be so rare as might be assumed from the literature Contrary to what might have been expected from the common notion of the relative incidences the solitary variant even outnumbered the disseminated form of the disease Solitary mastocytoma has been described as constituting 10–15 of all cases of mastocytosis (19) No reliable figures are available on the total incidence of masto cytosis The incidence reported here presumably comes close to the true incidence The dis



Fig. 7. Solitary mastocytoma on left forearm. Positive Darier's sign. Erythema covers major part of forearm.

s mutated form can hardly pass unnoticed, and practically all cases of the solitary variant with its fairly conspicuous symptoms must be detected though the lesions may sometimes be mistaken for a common nevus or some other skin disease. A positive Darier's sign was the clue to the diagnosis in all 8 cases. But this sign is not conclusive since a few other cases with a suspected Darier's sign were observed where biopsy failed to confirm the diagnosis. Yet it must be borne in mind that even histological examination may sometimes miss mast cells undergoing degranulation.

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the other hand as in Caplan's series (9) the observed frequency of allergic disorders among such members was higher than that expected

Two patients had general symptoms One patient exhibited a general flush precipitated by mechanical irritation of the lesion This patient had an increased urinary excretion of histamine General flushing is characteristic of urticaria pigmentosa and also of the solitary variant (2 3 7 12 23 25) The flush is generally believed to be induced by the release of histamine into the circulation from the tumour

Cases of urticaria pigmentosa with bronchial asthma are known (24) Histamine can produce bronchial constriction in individuals with bronchial asthma or bronchitis but has only negligible effect on the bronchioles in normals (11)

Table 2 *Results of laboratory investigation*

Case	Histamine excretion µg/kg body weight/24 h	5 HIAA excretion mg/g creatinine	Coagulation studies
1	8	17 9	Normal
2	0 4	0 3 mg/24 h	Normal
3	—	21 4	Normal
4	0 3–0 4	2 mg/24 h	Normal
5	0 4–0 5	8 2 14 3	Fibrinogen 0 13 g/100 ml No local fibrinolysis in tumour
6	0 4–0 5	6 5	Normal

Table 3 *Results of histamine studies in case no 1 (spectrophotometric method)*

Time (h)	Collected volume (ml)	Histamine in collected volume (µg)	Methylhistamine in collected volume (µg)
24	145	54	161
8	50	16	29
16	125	35	66



Fig 3 Collections of mast cells in upper dermis with concentrations around excretory ducts of sweat glands and around hair follicles Cells contain numerous metachromatic granules in sections stained with toluidine blue (case no 2) Toluidine ×75 (Alf Rausing)

One patient (no 4) who had expiratory wheezing on stimulation of the tumour obviously had a disposition to asthmatic disease

One of the patients in the material had an apparently coincidental convulsive disorder Such cases have also been described earlier (22) Of Cole's (10) fourteen patients with urticaria pigmentosa two had seizures without apparent causal connection Seizures in an adult with mast cell infiltration of the meninges as a part of systemic mastocytosis have been reported (26) In this case nothing suggested such an event The histamine shock of urticaria pigmentosa has been associated with loss of consciousness but not with seizures (5 33)

The risk of systemic involvement is much less when mastocytosis appears in childhood than when it does not occur until later in life (34) Particularly the diffuse or bullous forms are prone to be associated with internal disturbances (8) In none of the present cases of solitary mastocytoma did clinical examination skeletal X ray or investigation of the bone marrow reveal any signs of systemic involvement Visceral and skeletal disturbances have been

suspected in solitary mastocytosis (7 15) but have not been demonstrated with certainty

Increased amounts of histamine in the urine have been found in most but not in all patients with urticaria pigmentosa The histaminuria is probably due to overproduction of histamine by the increased number of mast cells in mastocytosis and not to an increased rate of histamine formation from histidine or to impaired catabolism of histamine (13)

Only two cases of solitary mastocytoma seem to have been studied with reference to histamine excretion and in both it was increased (7 14) In the present investigation it was normal in four cases and substantially increased in one (Tables 2 and 3) Normal values for methylhistamine are lacking but they are probably 10 times as high as the corresponding values for histamine (about 0 3 μg/kg/24 hours) (36) Anyway there is reason to assume that the values in case 1 were substantially increased The increase cannot be explained by the use of the spectrophotometric method though it does tend to yield higher values Nor can it be explained on dietary grounds (16) At least in cases 1 4 and 5 the tumour was of similar appearance and size and none of these children showed any evidence of internal lesions It is therefore difficult to explain the variation in histamine excretion There was no further increase of the histamine concentration in urine collected during mechanical stimulation of the tumour and administration of aspirin to the patient

The urinary excretion of 5 HIAA was normal (in controls it ranged from 8–40 mg/g creatinine)

A tendency to haemorrhage is a well known but not very common characteristic of urticaria pigmentosa It has been difficult however to correlate the bleeding tendency with the results of studies of the coagulation mechanism (18) In the present series of solitary mastocytoma laboratory studies showed no disturbances of the coagulation mechanism and particularly no abnormal amounts of a circulating anticoagulant of heparin type Neither was there any signs of local fibrinolytic activity in the tumour and the general fibrinolytic activity in the circulation was normal

According to the literature the prognosis is good and in most cases the condition disappears spontaneously (9 19 20) Though the outlook is thus bright the tumour has often been a matter of great concern to the mother Such concern can however be dispelled by early recognition of the condition and reassurance of the parents of the innocuous nature of the lesion

SUMMARY

Eight cases of solitary mastocytoma among children born in 1966–1968 in the city of Malmo have been recorded Six of them were submitted to investigation The diagnosis was confirmed by histological examination In most cases the tumour appeared at birth or soon after No signs of systemic mastocytosis were revealed by X ray of the skeleton or by bone marrow studies

One patient had an attack of generalized flushing and another had an attack of bronchial asthma precipitated by mechanical stimulation of the tumour In the first case there was an increased urinary excretion of histamine while it was normal in all the others The coagulation mechanism was normal and particularly no pathological amounts of a circulating anticoagulant of heparin type were demonstrated

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REFERENCES

1 Bigelow E L Changing concepts concerning mastocytosis *J Pediat* 58 499 1961
2 Birt A R & Nickerson M Generalized flushing of the skin with urticaria pigmentosa *Arch Derm* 80 311 1959
3 Blaich, W Solitares tuberoses Mastocytom mit generalisierten Gefasskrisen *Z Haut Geschlechtskr* 28 326 1960
4 Bloom F Spontaneous solitary and multiple mast cell tumours (mastocytoma) in dogs *Arch Path* 33 661 1942

5 Bloom G Dunér H Pernow B Winberg J & Zetterstrom R Spontaneous histamine shocks in urticaria pigmentosa *Acta Paediat* 47 152 1958
6 Brett E M Ong B H & Friedmann T Mast cell disease in children *Brit J Derm* 79 197 1967
7 Brogren N Duner H Hamrin B Pernow B Theander G & Waldenstrom J Urticaria pigmentosa (mastocytosis) A study of nine cases with special reference to the excretion of histamine in urine *Acta Med Scand* 163 223 1959
8 Burgoon C F Jr Graham J H & McCaffree D L Mast cell disease *Arch Derm* 98 590 1968
9 Caplan R M The natural course of urticaria pigmentosa *Arch Derm* 87 146 1963
10 Cole A R C Mastocytosis Report of a case in a 15 ½-week old infant with cutaneous and generalized skeletal changes *Arch Dis Child* 40 677 1965
11 Curry J J The action of histamine on the respiratory tract in normal and asthmatic subjects *J Clin Invest* 25 785 1946
12 Degos R Delort J Verliac I & Civatte F Mastocytose à plaque unique du nourrisson Poussées bulleuses locales associées à une vaso dilatation generalisée des téguments *Bull Soc Franc Derm Syph* 62 135 1955
13 Demis D J The mastocytosis syndrome Clinical and biological studies *Ann Intern Med* 59 194 1963
14 Demis D J Walton M D & Higdon R S Histaminuria in urticaria pigmentosa and the mastocytosis syndrome A clinical study and review of the recent literature with definition of the mastocytosis syndrome *Arch Derm* 83 127 1961
15 Graciansky M P Loewe Lyon Grupper C & Taieb R Mastocytome unique avec poussee bulleuse *Bull Soc Franc Derm Syph* 61 104 1954
16 Granerus G Urinary excretion of histamine methylhistamine and methylimidazoleacetic acids in man under standardized dietary conditions *Scand J Clin Lab Invest* 22 Suppl 104 59 1968
17 Gross P Urticaria pigmentosa (solitary lesion) *Arch Derm* 29 451 1934
18 Johannsen L & Kunzer W Zum Verhalten der Blutgerinnung bei Urticaria Pigmentosa *Arch Kinderheilk* 174 133 1966
19 Johnson W & Helwig E Solitary mastocytosis *Arch Derm* 84 806 1961
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Table 1 *Age of the patients and time of the two recordings For every record percentage of REM sleep time and the values for the ratio stage 2/stage 3 + 4 and stage 2 + 3/stage 4 are given The last two columns give the clinical diagnosis and the findings of the routine EEG*

Subjects age	Days after injury	Percentage of REM sleep	Stage 2 / stage 3 + 4	Stage 2 + 3 / stage 4	Diagnosis	Routine EEG findings
L D	2	30	0 09	5 05	Fracture of skull concussion	Right occ slowing
2 mo	10	43	0 07	11 80		
M K	3	42	0 19	33 50	Fracture of skull	Right par -occ depression
6 mo	11	36	0 11	11 70		
O B	2	13	1 75	36 00	Depressed orbital fracture operated on 3rd day	Right par -occ slowing
6 mo	10	17	0 69	30 14		
D R	0	26			Fracture of skull concussion chronic brain damage	General depression of amplitude already before injury
9 mo	7	18				
M N	4	30	0 09	10 70	Fracture of skull	Normal
9 mo	12	20	0 02	7 70		
C Z	1	11	0 53	12 22	Concussion	Normal
10 mo	22	2.	0 22	8 09		
A S	4	7	0 48	6 70	Depressed right par fracture	Normal
1 y	13	21	0 43	6 10		
A W	4	18	1 71	11 96	Fracture of skull	Normal
1 y	9	9	0 53	8 63		
U B	1	23	0 48	4 15	Fracture of skull	Normal
1 y	10	24	0 14	3 10		
M F	0	14	2 60	34 00	Concussion	Normal
1 y	8	27	1 14	5 22		
R Z	2	17	2 27	7 00	Concussion	Normal
2 y	16	11	0 69	2 60		
A S	1	13	1 21	39 10	Fracture of skull concussion	Normal
2 / y	11	13	0 21	9 50		
M S	1	7	0 91	4 16	Contusion transient aphasia + paresis right arm	Normal
2 / y	11	3	0 11	1 06		
P S	5	23	0 67	3 36	Concussion	Dysrhythmic
2 / y	13	20	0 39	1 80		
M W	2	22	1 40	∞	Contusion seizure with mouth movements and gaze to left side	Dysrhythmic
3 y	9	31	0 50	5 40		

ings taken soon after the accident In order to investigate whether there were additional changes in the amount of spindles per time unit and in the duration of spindles interval and duration histograms of the spindles during two epochs of NREM sleep were made From these histograms (an example is given in Fig 2) the median was computed the median being preferred to the mean value because of the marked asymmetry of some distributions This rather cumbersome procedure was carried out in the first 10 patients studied and the results are given in Table 2 Since no relation has been found between the side of clinical symptoms and the median values for spindle intervals or spindle duration of one hemisphere the mean value for both hemispheres has been used for statistical computation The intervals were shorter during the acute stage after the injury in all 10 patients Less consistent changes were observed in the duration of the spindles Seven out of ten patients showed an increase in spindle duration after the injury while in 3 patients a decrease was found When there was an increase however the median value was considerably higher in the first record when compared with the second while in those subjects who had shorter spindles in the first record only a slight decrease was found in the second Using Wilcoxon's matched—pairs signed—rank's test p was found to be less than 0 05 and greater than 0 02 in two—tailed test

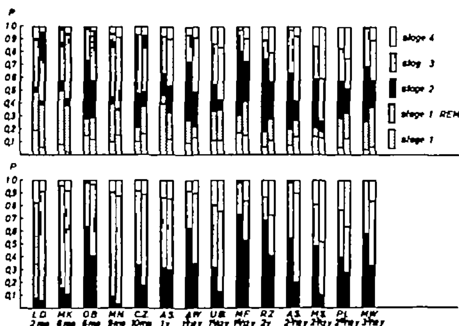


Fig 1 Upper row Probability density for the different sleep stages during the acute stage after the injury (left column) and during the control record 1 3 weeks later (right column) Lower row Probability density for the different stages of NREM sleep Note increase of stage 2 and reduction of stage 4 in the record following the trauma

served during the recording and behavioural patterns such as gross movements mouthing and vocalization were noted on the record

For every page of the recording (20 sec) the sleep stage was classified according to the criteria of Dement & Kleitman (7) Only 20 sec periods with at least one eye movement were classified as REM sleep The number of eye movments per minute during REM slep was counted from the electro oculographic recording In the leads F_3–C_3 and F_4–C_4 of the EEG the duration of the sleep spindles and the duration of the intervals between the beginning of one spindle and the beginning of the next were measured with a ruler A spindle was defined as a regular sequence of 11–14 cps waves of at least 4 mm (4/15 sec) duration Intervals longer than 100 sec and those during which a gross body movement occurred were not counted

RESULTS

1 *Quantitative changes of sleep cycles*

In one patient (U R) a child with severe chronic brain damage the EEG showed low voltage and high frequency continuously during NREM sleep in both recordings and it was not possible to discriminate the different stages In the remaining 14 patients an increase of stage 2 (light sleep) and a relative decrease of the stage 3 and 4 (intermediate and deep sleep) were shown during NREM sleep in the first recording made after the injury During this acute stage 13 patients had a higher percentage of the NREM stages with spindles (stages 2 and 3) and a lower percentage of stage 4 which is characterized by high amplitude slow activity and an absence of spindles Only in the youngest patient aged 2 months initially was the opposite pattern found i e more stage 4 during the first recording Since sleep spindles only appear in the EEG between the second and third month of life the increase of spindle stages in the second recording in this patient may have been due to the fact that the normal developmental changes exceeded the changes caused by the trauma

No consistent changes of total REM sleep time have been found In 9 patients a higher percentage of REM sleep was found in the first record and in 6 patients more REM sleep was found during the second record

Fig 1 shows the probability density of the different sleep stages for the two recordings in 14 patients In Table 1 the percentages of REM sleep time and the values for the ratio stage 2/stages 3+4 and stages 2+3/stage 4 are shown together with the clinical findings in all subjects

2 *Changes of spindle activity*

As mentioned earlier an increase of NREM sleep stages with spindles and a relative decrease of stage 4 was found in the first record

Table 1 *Age of the patients and time of the two recordings For every record percentage of REM sleep time and the values for the ratio stage 2/stage 3+4 and stage 2+3/stage 4 are given The last two columns give the clinical diagnosis and the findings of the routine EEG*

Subjects age	Days after injury	Percentage of REM sleep	Stage 2 / stage 3+4	Stage 2+3 / stage 4	Diagnosis	Routine EEG findings
L D	2	30	0 09	5 05	Fracture of skull concussion	Right occ slowing
2 mo	10	43	0 07	11 80		
M K	3	42	0 19	23 50	Fracture of skull	Right par -occ depression
6 mo	11	36	0 11	11 70		
O B	2	13	1 73	36 00	Depressed orbital fracture operated on 3rd day	Right par -occ slowing
6 mo	10	17	0 69	30 14		
U R	0	26			Fracture of skull concussion chronic brain damage	General depression of amplitude already before injury
9 mo	7	18				
M N	4	30	0 09	10 70	Fracture of skull	Normal
9 mo	12	20	0 02	7 70		
C Z	1	11	0 33	12.22	Concussion	Normal
10 mo	22	22	0.22	8 09		
A S	4	7	0 48	6 70	Depressed right par fracture	Normal
1 y	13	21	0 43	6 10		
A W	4	18	1 71	11 96	Fracture of skull	Normal
1 y	9	9	0 53	8 63		
U B	1	23	0 48	4 15	Fracture of skull	Normal
1 / y	10	24	0 14	3 10		
M F	0	14	2.60	34 00	Concussion	Normal
1 / y	8	27	1 14	5 22		
R Z	2	17	2.27	7 00	Concussion	Normal
2 y	16	11	0 69	2 60		
A S	1	13	1.21	39 10	Fracture of skull concussion	Normal
2 / y	11	13	0 21	9 50		
M S	1	7	0 91	4 16	Contusion transient aphasia + paresis right arm	Normal
2 / y	11	5	0 11	1 06		
P S	5	23	0 67	3 36	Concussion	Dysrhythmic
2 y	13	0	0 39	1 80		
M W	2	22	1 40	∞	Contusion seizures with mouth movements and gaze to left side	Dysrhythmic
3 / y	9	31	0 30	5 40		

ings taken soon after the accident In order to investigate whether there were additional changes in the amount of spindles per time unit and in the duration of spindles interval and duration histograms of the spindles during two epochs of NREM sleep were made From these histograms (an example is given in Fig 2) the median was computed the median being preferred to the mean value because of the marked asymmetry of some distributions This rather cumbersome procedure was carried out in the first 10 patients studied and the results are given in Table 2 Since no relation has been found between the side of clinical symptoms and the median values for spindle intervals or spindle duration of one hemisphere the mean value for both hemispheres has been used for statistical computation The intervals were shorter during the acute stage after the injury in all 10 patients Less consistent changes were observed in the duration of the spindles Seven out of ten patients showed an increase in spindle duration after the injury while in 3 patients a decrease was found When there was an increase however the median value was considerably higher in the first record when compared with the second while in those subjects who had shorter spindles in the first record only a slight decrease was found in the second Using Wilcoxon s matched—pairs signed—ranks test p was found to be less than 0 05 and greater than 0 02 in two—tailed test

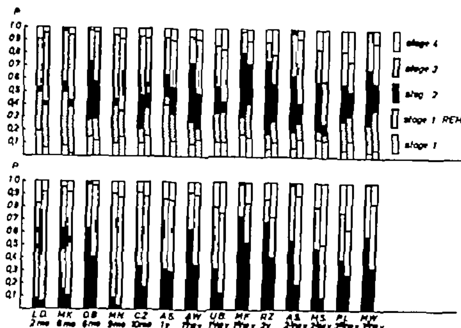


Fig 1 Upper row Probability density for the different sleep stages during the acute stage after the injury (left column) and during the control record 1-3 weeks later (right column) Lower row Probability density for the different stages of NREM sleep Note increase of stage 2 and reduction of stage 4 in the record following the trauma

served during the recording and behavioural patterns such as gross movements mouthing and vocalization were noted on the record

For every page of the recording (20 sec) the sleep stage was classified according to the criteria of Dement & Kleitman (7) Only 20 sec periods with at least one eye movement were classified as REM sleep The number of eye movments per minute during REM slep was counted from the electro oculographic recording In the leads F_4-C_4 and F_3-C_3 of the EEG the duration of the sleep spindles and the duration of the intervals between the beginning of one spindle and the beginning of the next were measured with a ruler A spindle was defined as a regular sequence of 11-14 cps wave of at least 4 mm (4/15 sec) duration Intervals longer than 100 sec and those during which a gross body movement occurred were not counted

RESULTS

1 *Quantitative changes of sleep cycles*

In one patient (U R) a child with severe chronic brain damage the EEG showed low voltage and high frequency continuously during NREM sleep in both recordings and it was not possible to discriminate the different stages In the remaining 14 patients an increase of stage 2 (light sleep) and a *relative decrease* of the stage 3 and 4 (intermediate and deep sleep) were shown during NREM sleep in the first recording made after the injury During this acute stage 13 patients had a higher percentage of the NREM stages with spindles (stages 2 and 3) and a lower percentage of stage 4 which is characterized by high amplitude, slow activity and an absence of spindles Only in the youngest patient aged 2 months initially was the opposite pattern found i e more stage 4 during the first recording Since sleep spindles only appear in the EEG between the second and *third month of life the increase of spindle* stages in the second recording in this patient may have been due to the fact that the normal developmental changes exceeded the changes caused by the trauma

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2 *Changes of spindle activity*

As mentioned earlier an increase of NREM sleep stages with spindles and a relative decrease of stage 4 was found in the *first record*

bral trauma the brain stem and in particular the mesencephalo-diencephalic structures represent the most vulnerable region where all lines of force must converge Neurophysiological evidence for this assumption is provided by experiments with monkeys carried out by Ward (24) after concussion the activity recorded from the brain stem reticular formation showed far greater and longer depression than that recorded from cortical and other subcortical structures Since the most important structures for the regulation of sleep cycles are located in the brain stem it was not surprising to find almost regularly that transient alterations of sleep patterns after head injuries occurred even in cases in which symptoms were only mild and no EEG alterations could be detected by routine visual analysis

For clinical purposes these changes in sleep pattern are of limited value during the acute stage after the injury The alterations are only slight and the inter and intraindividual variability in the duration of sleep stages and cycles may be considerable (21 23) Moreover a remarkably large number of children suffering from head injury are "high risk" children coming from low social classes presenting an anamnesis of previous trauma or of other cerebral disturbances (12) In these children one EEG after an accident is difficult to evaluate unless previous recordings are at hand The need for a dynamic interpretation of the post traumatic EEG stressed by Kellaway (14) and Rodin (20) exists also for the evaluation of polygraphic sleep recordings Our findings show that a child in whom symtoms have disappeared and the EEG appears normal may not be recovered completely after a head injury Functional disturbances in certain structures of the brain stem may still persist and we consider that a cautious approach in the management of these patients is warranted

The results of our study—reduction of sleep stages with highly synchronized EEG activity increase in amount and duration of sleep spindles and increase in the amount of rapid eye movements per time unit after acute head injury—may give rise to some thoughts about the pathophysiology of head traumas An increase of stage 2 sleep with reduction of the percentage of stage 3 and stage 4 sleep has been observed by Adey *et al* (1) in patients with chronic high cervical lesions These authors suggest that the reason for these changes might be a decreased proprioceptive input The same changes in the percentage of NREM stages have been seen by Bergamasco *et al* (2) in those patients with post traumatic coma who showed sleep—like cyclic fluctuations of the EEG pattern Interesting results have been obtained by Weitzman *et al* (25) in monkeys following the administration of *p*-chlorophenylalanine a selective depletor of serotonin which caused not only a reduction of total NREM time but increased values for the ratio stages 1+2 to stage 3+4 as well Sachs (22) found that the cerebral spinal fluid (C S F) level of serotonin rose in both animals and man after concussion This suggests that an increased flow of serotonin from the brain into the C S F may occur thus diminishing the content of serotonin in the serotonergic neurons in the raphe nuclei of the brain stem which would explain the relative increase in light sleep following head injuries

In our patients we were unable to demonstrate a significant changes of the percentage of REM sleep time as described by Adey *et al* (1) and by Bergamasco *et al* (3) The structures responsible for the organization of REM sleep in the nucleus reticularis pontis are perhaps less susceptible to traumatic lesions which could be of biological value regarding the importance of the REM sleep for the organism as shown in sleep deprivation experiments The *relatively* mild trauma sustained in most of our patients may not have been sufficient to alter REM sleep to a degree detectable by our methods We have however studied one child (which could not be included in our study because we were unable to obtain a second control recording) with neurological symptoms of brain stem damage who had no REM sleep *during* more than three hours of recording

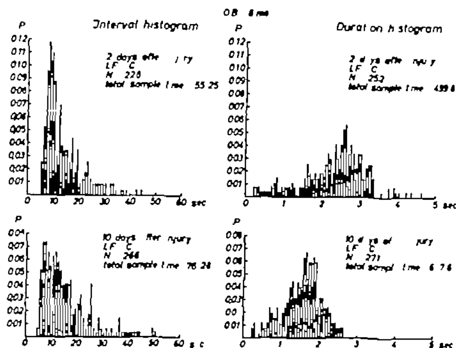


Fig 2 Example for interval and duration histograms of sleep spindles The intervals between the spindles are shorter and the spindles are longer during the acute stage after the head trauma

ing which suggests a strong tendency of the spindle length to increase after an acute head injury

The frequency of the waves within the spindles was 13–14 cps in both recordings in all subjects

3 *Changes in the amount of rapid eye movements during REM sleep*

Whereas the time spent in REM sleep and the percentage of REM sleep in total sleep time did not show consistent changes after the injury the number of rapid eye movements was higher in 12 out of 14 patients (Table 3) In one patient (A SZ) the eye movements could not be counted because of a technical artefact in one record The increase in the amount of eye movements is significant at the 1% level (Wilcoxon matched—pairs signed—ranks test two tailed)

DISCUSSION

Denny Brown & Russel (8) concluded from animal experiments that in closed cranial-cere

Table 2 *Median of length of the intervals between spindles (left column) and median of spindle duration (right column) during two epochs of NREM sleep in the record shortly after the trauma and in the control record*

Subject	Spindle intervals sec		Spindle duration sec	
	1st record	2nd record	1st record	2nd record
M K	10 95	12 65	1 55	1 43
O B	11 35	13 45	2 52	1 57
U R	10 10	13 70	2 04	1 76
M N	11 75	13 15	1 56	1 47
C Z	11 10	11 50	1 45	1 49
A S	12 60	15 65	0 78	0 79
U B	14 50	28 55	1 22	0 90
M F	15 10	21 85	0 75	0 54
A S	14 55	18 45	1 06	0 94
M S	19 45	23 50	0 62	0 66

Table 3 *Mean number of rapid eye movements per minute in the record following the trauma (left column) and the control record (right column)*

Subject	1st record	2nd record
L D	17 0	14 8
M K	28 8	21 2
O B	12 9	7 9
U R	26 1	24 6
M N	27 1	10 1
C Z	10 1	6 6
A S	6 2	4 9
A W	16 5	9 9
U B	16 2	12 4
M F	15 6	19 2
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11–14 cps activity indistinguishable from sleep spindles has been seen in patients with post traumatic coma by Chatrian *et al* (5) Bergamasco *et al* (3) and Lucking (17) have described the gradual reappearance of sleep spindles in the EEG of patients recovering from profound posttraumatic coma None of these authors offer an explanation for these findings We also are unable to give more than a speculative explanation for the slight but consistent increase of spindle activity in our patients Because of the statistical properties of spindles and REM in normal infants we have hypothesized that both phenomena might be the result of random noise in certain brain structures in the absence of patterning sensory inflow (16) Pre and postsynaptic inhibition of various afferents during bursts of REM has been demonstrated in animal experiments whereas only indirect indications exist for the occurrence of such a mechanism during spindle discharges Experimental data by Foltz & Schmidt (9) suggest a failure in conduction of peripheral afferent stimulation through the brain stem after concussion If our hypothesis on the mechanism of spindles and REM holds true such an afferent inhibition could be the cause of the increase in spindles and REM after head injuries

SUMMARY

The first two cycles of normal night sleep were polygraphically recorded in 15 infants and young children during the acute stage after head injuries Control records were carried out 1–3 weeks later

Following the trauma there was an increase in stage 2 sleep and a relative reduction of the stages 3 and 4 During NREM sleep the percentage of stages with spindles (2 and 3) was higher than that of stage 4 No consisteunt changes in the percentage of REM sleep could be demonstrated

The intervals between spindles were shorter after the trauma and in most cases the duration of spindles was longer The amount of rapid eye movements per minute was higher during the acute stage The implications for the pathophysiology of head injuries in young infants are discussed

ACKNOWLEDGEMENTS

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REFERENCES

1 Adey W R Bors E & Porter R W EEG sleep patterns after high cervical lesions in man *Arch Neurol* 19 377 1968
2 Bergamasco B Bergamini L & Doriguzzi T Clinical value of the sleep electroencephalographic pattern in post traumatic coma *Acta Neurol Scand* 44 495 1968
3 Bergamasco B Bergamini L Doriguzzi T & Fabiani D EEG sleep patterns as a prognostic criterion in post traumatic coma *Electroenceph Clin Neurophysiol* 24 374 1968
4 Bergamasco B Doriguzzi T Sacerdote I & Chiono C Registrazioni poligraphiche notturne in soggetti in coma post traumatico *Boll Soc Ital Biol Sper* 43 322 1966
5 Chatrian G E White L E & Daly D Electroencephalographic patterns resembling those of sleep in certain comatose states after injuries to the head *Electroenceph Clin Neurophysiol* 15 272 1963
6 Creissard P Chodkiewicz J P & Thebault A M A propos de cent cas de traumatisme cranio cérébral chez le nourisson *Neuro Chir* (Paris) 14 759 1968
7 Dement W & Kleitman N Cyclic variations in EEG during sleep and their relations to eye movements body motility and dreaming *Electroenceph Clin Neurophysiol* 9 673 1957
8 Denny Brown D & Russel J Experimental cerebral concussion *Brain* 64 93 1941
9 Foltz E L & Schmidt R P The role of the reticular formation in the coma of head injury *J Neurosurg* 13 145 1956
10 Frantzen E Harvald B & Haugsted H Fresh head injuries *Acta Psych Scand* 33 417 1958
11 Harris P Head injuries in childhood *Arch Dis Child* 32 488 1957
12 Hjern B & Nylander I Acute head injuries in children *Acta Paediat Scand* 53 Suppl 152 1 1964
13 Jouvet M Pellin B & Mounier D Polygraphic study of the diferent sleep phases during chronic disturbances of consciousness (prolonged comas) *Electroenceph Clin Neurophysiol* 14 138 1962

14 Kellaway P Head injuries in children *Electroenceph Clin Neurophysiol* 7 497 1955
15 Lenard H G EEG Veraenderungen bei frischen Schadeltraumen im Kindesalter *Munch Med Wschr* 107 1820 1965
16 — The development of sleep spindles in the EEG during the first two years of life *Neuropadiatrie* *1* 264 1970
17 Luckhe C M *Klinische und elektroencephalographische Befunde des Hirntodes nach schweren Hirnverletzungen* Paper read at the annual meeting of the German EEG Society Bonn 1969
18 Melchior J The incidence of head injuries in children *Acta Paediat Scand* 50 47 1961
19 Passouant P Cadilhac J Delange M Baldy Moulinier M & El Kassabgui N E Différents stades électriques et organisation en cycle des comas post traumatiques. Enregistrement polygraphique de longue durée *Rev Neurol* 111 391 1964
20 Rodin E A Contribution of the EEG to prognosis after head injury *Dis Nerv Syst* 28 595 1967
21 Roffwarg H P Muzio J N & Dement W C Ontogenetic development of the human sleep-dream cycle *Science* 152 604 1966
22 Sachs E Acetylcholine and serotonin in the spinal fluid *J Neurosurg* 14 22 1957
23 Stern E Parmelee A H Akiyama Y Schultz, M A & Wenner W H Sleep cycle characteristics in infants *Pediatrics* 43 65 1969
24 Ward A A Physiological basis of concussion *J Neurosurg* 15 129 1958
25 Weitzman E D Rapport M M McGregor P & Jacoby J Sleep patterns of the monkey and brain serotonin concentration Effect of *p*-chlorophenylalanine *Science* 160 1361 1968

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5 Chatrian G E White L E & Daly D Electroencephalographic patterns resembling those of sleep in certain comatose states after injuries to the head *Electroenceph Clin Neurophysiol 15* 272 1963

6 Cressard P Choulkiewicz J P & Thébault A M A propos de cent cas de traumatisme cranio cérébral chez le nourisson *Neuro Chir* (Paris) *14* 759 1968

7 Dement W & Kleitman N Cyclic variations in EEG during sleep and their relations to eye movements body motility and dreaming *Electroenceph Clin Neurophysiol 9* 673 1957

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sleep occurs in all mammalian species from man to the opossum

In sleeping human infants Zipperling (150) observed as early as 1913 repeatedly occurring periods of REM with irregular respiration and body motility but the significance of this observation was not understood until Kleitman & Engelman (77) and Aserinsky & Kleitman (6) reported the finding of an alternating sleep pattern also in the very young age group Since then meticulous behavioural observations (10 148 149) and comprehensive polygraphic investigations (25 34 35 37 38 39 40 53 91 102 103 104 108 109 110 115 116 132) have been carried out on sleeping prematures and full term newborns Fewer reports have been published about the sleep pattern of infants and older children (47 79 128 141)

NREM SLEEP

Synonyms for NREM sleep are regular sleep (148) quiet sleep (31 101) ruhiger Schlaf (66 119) sommeil calme (91) state 1 (114) In the neurophysiological literature one finds the terms synchronized sleep (68) high voltage slow wave sleep (26) telencephalic sleep (67) Both deep sleep" and light sleep have been used for the description of this sleep state and it is obvious that these terms must be discarded in order to avoid misunderstandings

During NREM sleep the infant is lying quiet except for irregularly occurring sudden generalized movements called startles Startles are not identical with the Moro response they occur without external stimulation and they do not consist of a regular pattern of muscular activity (114) After the newborn period startles become rare Tonic activity can be recorded from the submental muscles this phenomenon does not always occur especially in very young infants (41) Both respiratory rate and heart rate are slow and regular (116) the systolic blood pressure is low and stable (55) There are no movements of the eye balls after the newborn period slow regular rolling movements may occur at the onset of the first sleep cycle (27)

The EEG of NREM sleep undergoes marked developmental changes Until 44–46 weeks conceptional age the dominating pattern is the so called tracé alternant (131) consisting of longer periods with low voltage activity interrupted by periods of irregular high delta and theta waves 1–4 sec long The flat periods which may show even a zero line EEG before 32 weeks gestational age increase in amplitude with increasing age The bursts of the tracé alternant are mixed with low amplitude beta activity at 32 weeks gestational age with sharp waves at 36 weeks and are made up of bilaterally synchronous delta and theta waves at term During the second month after term the tracé alternant is replaced by a sequence of EEG patterns corresponding to the NREM stages of adults classified by Dement & Kleitman (28) stage 1 any pattern between wakefulness and the appearance of 12–14 cps spindles stage 2 low voltage EEG with spindles and occasional slow waves stage 3 intermediate stage with high amplitude slow activity and spindles stage 4 high amplitude delta and sub-delta waves without spindles Changes in the relative amount of NREM stages during the first years of life are mainly due to a change in the amount of spindles This peculiar EEG pattern appears during the second month of life spindles are longest and most frequent during the third and fourth month thereafter they decrease in amount and length until they are restricted to stage 2 at the beginning of a NREM epoch (83) This development results in a continuous decrease of stage 3 and a relative increase of stage 4 while there is little change in the amount of stage 2

In animals (7 52 81) human adults (57) and in human infants (58 84 117 120) proprioceptive reflexes undergo no or only little depression during NREM sleep as compared to wakefulness This is also the case for the Moro reflex in infants Exteroceptive reflexes and the vestibulo-ocular response (14 123) on the other hand are markedly depressed or disap-

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REVIEW ARTICLE

SLEEP STUDIES IN INFANCY

Facts Concepts and Significance

H G LENARD

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Although infants and young children spend the major part of their time sleeping pediatricians have for a long time shown surprisingly little interest in the physiology and pathology of sleep Traditional concepts were handed over almost unchanged from one generation of pe diatricians to the next Sleep was simply re- garded as the opposite of wakefulness as a period of recreation for the organism (tropho- tropic phase) in which all vegetative and neural systems were switched to a lower level of ac tivity The large amount of sleep required in the young though no longer contributed to anoxia of the infantile brain caused by exces- sive intake of milk (20 122) was explained by the greater fatigueability of the immature brain

Immaturity and sleep are closely related Those brain centers which just started functioning tend to relapse into the state they were in before being engaged into brain function (107) Dur ing the last 30 years however studies of theore tical and clinical neurophysiologists psycholo- gists psychiatrists and a few pediatricians have lead to such a wealth of new information that the 4377 references in Kleitman s famous book Sleep and Wakefulness (76) are today no long- er a complete bibliography Recent research has shown the possibilities of applied sleep research especially in young infants It therefore seems justified to review the results of recent sleep research to line out the present concept of sleep and to discuss their possible relevance for the pediatrician the pediatric neurologist and the student of human development

THE DUALISTIC NATURE OF SLEEP

When the pioneers of electroencephalography found different patterns of bioelectric brain ac tivity during sleep (86) they attributed this find ing to different levels of sleep depth In 1953 Aserinsky & Kleitman (4) found that a peculiar EEG pattern resembling that of wakefulness occurred periodically during sleep and coin cided with rapid eye movements (REM) under the closed lids During the epochs with REM the subjects showed various forms of motor ac tivity (28 30) heart rate and respiratory rate increased (3) and the tonic activity of the anti gravity muscles disappeared (11 63) The REM epochs were found to be closely related to the subjective experience of dreaming (5 29) These discoveries together with the results of neurophysiological studies—especially of Jou vet s group at Lyon and of Pompeiano s group at Pisa—lead to the suggestion that sleep is not a quantitative continuum of decreasing and again increasing vigilance but that it consists of two different states of behavioural and neural organisation the sleep with REM (REM sleep) and the sleep without REM (NREM sleep) The alteration between REM sleep and NREM

sleep occurs in all mammalian species from man to the opossum

In sleeping human infants Zipperling (150) observed as early as 1913 repeatedly occurring periods of REM with irregular respiration and body motility but the significance of this observation was not understood until Kleitman & Engelman (77) and Aserinsky & Kleitman (6) reported the finding of an alternating sleep pattern also in the very young age group Since then meticulous behavioural observations (10 148 149) and comprehensive polygraphic investigations (25 34 35 37 38 39 40 53 91 102 103 104 108 109 110 115 116 132) have been carried out on sleeping prematures and full term newborns Fewer reports have been published about the sleep pattern of infants and older children (47 79 128 141)

NREM SLEEP

Synonyms for *NREM* sleep are regular sleep *(148)* quiet sleep (31 101) ruhiger Schlaf (66 119) sommeil calme (91) state 1 (114) In the neurophysiological literature one finds the terms synchronized sleep (68) high voltage slow wave sleep (26) telencephalic sleep (67) Both deep sleep and light sleep have been used for the description of this sleep state and it is obvious that these terms must be discarded in order to avoid misunderstandings

During NREM sleep the infant is lying quiet except for irregularly occurring sudden generalized movements called startles Startles are not identical with the Moro response they occur without external stimulation and they do not consist of a regular pattern of muscular activity (114) After the newborn period startles become rare Tonic activity can be recorded from the submental muscles this phenomenon does not always occur especially in very young infants (41) Both respiratory rate and heart rate are slow and regular (116) the systolic blood pressure is low and stable (55) There are no movements of the eye balls after the newborn period slow regular rolling movements may occur at the onset of the first sleep cycle (27)

The EEG of NREM sleep undergoes marked developmental changes Until 44–46 weeks conceptional age the dominating pattern is the so called "trace alternant (131) consisting of longer periods with low voltage activity interrupted by periods of irregular high delta and theta waves 1–4 sec long The flat periods which may show even a zero line EEG before 32 weeks gestational age increase in amplitude with increasing age The "bursts of the trace alternant are mixed with low amplitude beta activity at 32 weeks gestational age with sharp waves at 36 weeks and are made up of bilaterally synchronous delta and theta waves at term During the second month after term the trace alternant is replaced by a sequence of EEG patterns corresponding to the NREM stages of adults classified by Dement & Kleitman (28) stage 1 any pattern between wakefulness and the appearance of 12–14 cps spindles stage 2 low voltage EEG with spindles and occasional slow waves stage 3 intermediate stage with high amplitude slow activity and spindles stage 4 high amplitude delta and sub delta waves without spindles Changes in the relative amount of NREM stages during the first years of life are mainly due to a change in the amount of spindles This peculiar EEG pattern appears during the second month of life spindles are longest and most frequent during the third and fourth month thereafter they decrease in amount and length until they are restricted to stage 2 at the beginning of a NREM epoch (83) This development results in a continuous decrease of stage 3 and a relative increase of stage 4 while there is little change in the amount of stage 2

In animals (7 52 81) human adults (57) and in human infants (58 84 117 120) proprioceptive reflexes undergo no or only little depression during NREM sleep as compared to wakefulness This is also the case for the Moro reflex in infants Exteroceptive reflexes and the vestibulo-ocular response (14 123) on the other hand are markedly depressed or disap-

pear during NREM sleep Nociceptive reflexes of infants, as the abdominal skin reflex or the Babinsky type foot sole reflex remain unchanged during both sleep states as does the arousal threshold to painful electrical stimuli in adults (84 113) Cortical evoked responses to stimuli of different modalities are higher in NREM sleep than in REM sleep (1 59 97 145) but this does not necessarily prove different degrees of reactivity since the amplitude of the evoked responses is partly a function of the amplitude of the background EEG

REM SLEEP

Synonyms for REM sleep are irregular sleep (148) active sleep (31 101) unruhiger Schlaf (119) sommeil actie (91) state 2 (114) D state (56 109) paradoxical sleep (69) desynchronized sleep (68) low amplitude high frequency sleep (26) rhombencephalic sleep (67) archeo sleep (68)

The most prominent feature of REM sleep is the occurrence of slow and rapid eye movements under the closed or partually opened lids REM are numerous in newborn infants and decrease in number during the first weeks of life In newborns they are present in an irregularly varying amount throughout the whole REM epoch after the third or fourth month of life they occur in bursts separated by periods without ocular motility It has been suggested that form and direction of the REM might have a relationship to dream imagery (30 126) but this hypothesis has been questioned because of statistical differences between the REM and the scanning eye movements during wakefulness (64 118) The occurrence of REM moreover in newborns animals and humans refutes at least a primary relation with the subjective experience of dreaming (95 98)

Several gross body movements usually leading to a change of position may occur during an epoch of REM sleep and myoclonic twitches and small movements of the extremities and the face can be observed The face movements may resemble psychologically meaningful facial expressions as for instance smiling or shuddering (65 143) No tonic activity can be recorded from the submental muscles

Respiratory rate and heart rate are higher and more irregular than during NREM sleep and may even be higher than during quiet wakefulness (116) Systolic blood pressure is also higher and shows greater fluctuations (55) Penile erections may occur though not as frequent in infants (80) as in adults (46)

The EEG of REM sleep consists of occasional small beta activity superimposed on high amplitude delta waves in prematures of 30 weeks gestational age Both types of waves disappear gradually so that at term we find a flat EEG which appears to be made up only of fast activity though spectral analysis (116) reveals a high percentage of delta and sub delta activity No important changes occur after the second or third month of life when the visible EEG pattern consists mainly of waves in the theta range

Other authors investigating human adults or carrying out experimental work with cats or monkeys reported identical results in REM sleep respiratory rate heart rate and systolic blood presure are higher and more irregular than during NREM sleep (3 48 75 141 147) Additional physiological data, though not obtained from studies in human infants appear to be important for the understanding of the functional organization of REM sleep In adults Mandell *et al* (87 88) found urine volume, osmolarity and 17 hydroxycorticoid output to be higher in REM sleep than in NREM sleep The oxygen consumption rate increases (18) and the intracranial pressure rises (24) From animal studies it is known that cerebral circulation is augmented by 30–50 (73 125) and cerebral temperature elevated (74 133) Proprioceptive reflexes are diminished or absent during REM sleep because of descending inhibitory influences on the fusimotor system (51 82) and on the spinal motoneurons (50 93) The firing rate of single neurons in various regions of the brain may be as high as during

wakefulness but is far more irregular (16 17 43 44 62) so that Evarts (42) suggests a disinhibition of inhibitory cortical interneurons which would involve a disorganisation of the cortical unit activity during REM sleep

There is growing evidence supporting the concept that REM sleep is not a steady state Besides "tonic mechanisms responsible for the desynchronization of the EEG and the inhibition of tonus in the antigravity muscles there are phasic components related in time to the REM bursts Respiratory and heart rate show an additional increase in frequency and irregularity (48 118) proprioceptive reflexes which are decreased during REM sleep show an additional phasic depression during REM bursts the myoclonic twitches coincide frequently with REM bursts and are probably the result of a phasic increase of neuronal activity in the pyramidal tract (2 49) Especially important is the finding of pre and postsynaptic inhibition occurring in various afferent pathways (8 9 13 15 21 94) because it may be causally related to the phasically occurring irregular phenomena

THE ONTOGENETIC DEVELOPMENT OF SLEEP CYCLES

In previable prematures from 24–27 weeks of gestational age Dreyfus Brisac (36) found no signs of a waking state but only atypical sleep which could not be classified as REM or NREM By means of EEG criteria the discrimination between the two sleep states is possible at a gestational age of about 29 weeks but the typical constellation of physiological parameters defining REM sleep appears rarely before the 34th week

Concerning the duration of the single sleep stages in newborns the data given by different authors are not quite comparable owing to differences in the criteria applied differences in recording conditions and some degree of interindividual variability (10 38 53 91 105 108 109 127) There would be agreement however about the following general facts During the first week of life the full term newborn sleeps 17–20 hours a day About 12 hours are spent in REM sleep 6–7 hours in NREM sleep and the rest of the time is distributed among the different waking activities The duration of one sleep cycle is 45–120 min NREM epochs of 10–20 min duration and REM epochs of 20–40 min duration alternate irregularly

Total sleep time decreases during the following months of life Parmelee *et al* (101) found an average sleep time of 17 8 hours during the first week of life and 14 3 hours at 35 weeks The relative amount of NREM sleep increases (22 33 127) the periodic alterations between the two sleep states become more regular After the second month of life sleep begins with an epoch of NREM sleep followed by the first REM epoch (frequently with very few eye movements) again followed by the NREM epoch of the second sleep cycle The NREM epochs show a gradual "building up beginning with Dement s stage 2 followed by stage 3 and stage 4 and end abruptly usually with a gross body movement then the REM epoch begins leading gradually to the next stage 2 ("saw tooth pattern of sleep cycles (116))

A very similar course of development has been found in all the mammalian species studied (22 23 90 130 138 144)

MECHANISMS AND FUNCTIONS OF SLEEP

The very complex neurophysiology of the sleep states has recently been reviewed in an excellent article by Jouvet (72) There the interested reader will find a detailed discussion of the present knowledge about structures and mechanisms responsible for the organisation of the two sleep states A possible monoaminergic theory of sleep has come into discussion during the last few years (70 78 99 124 146) Serotonergic neurons of the raphe complex and catecholaminergic neurons of the pontine tegmentum appear to be of crucial relevance *in* the triggering of the two states of sleep Increase in brain serotonin leads to an increase in

NREM sleep and blockage of monoaminooxydase leads to an elective suppression of REM sleep whilst the amount of REM sleep can be increased by reserpine releasing monoamines at the monoaminergic terminals This theory may be especially pertinent for the clinician since he will encounter metabolic disturbances leading to changes in the biogenic amines more often than localized lesions of brain structures responsible for the regulation of sleep states

Reviewing the large amount of new information on sleep one does not find any completely new aspect on the function of NREM sleep As a state in which all metabolic requirements are minimized it appears to be related to the need of the organism for rest and restitution The function of REM sleep on the other hand is still a matter of stimulating though largely hypothetical discussion

In an evolutionary theory of dreaming Snyder (140) suggests that REM sleep may have survival advantage under natural conditions by periodically decreasing the subjects threshold of arousal during sleep The weak point of this sentinel hypothesis seems to be that a lower arousal threshold for REM sleep is by no means an established fact The threshold of arousal is not in the first place dependent on he sleep state but on the properties of the stimulus Even for biologically meaningful stimuli the waking threshold may be higher in REM sleep than in NREM sleep (139) and potentially dangerous stimuli seem to produce reactions indepedently of state (84 13)

From a developmental point of view—taking into account the decreasing percentage of REM sleep with increasing age—Roffwarg *et al* (127) have hypothesized that the REM mechanism serves as an endogenous source of functional excitation necessary for the maturation of higher centers especially in utero and shortly after birth when exogenous stimulation is still scarce More specifically Berger (12) regards REM as a mechanism for the establishment of the neuromuscular pathways serving binocularly coordinated eye movements If we accept these concepts, we are still left with the problem of the functional significance of the other phasic phenomena occurring during REM sleep and of the cyclically occurring REM epochs in mature subjects—Another hypothesis (118) regards REM and the irregular fluctuations in many other parameters as the result of random noise in the CNS caused by a lack of patterning afferent input during the synchronously occurring inhibition of sensory afferents The decrease of REM sleep with the increase of the homeostatic capacities on the brain during ontogenesis (and the slight increase of REM sleep during old age (45 127)) would be in accordance with the hypothesis—The most recent hypothesis (100 142) suggests that alternating periods of ocular motility and ocular quiescence may not be specific to sleep but exist over all 24 hours of a day thus representing a general activity pattern of the brain

Though many problems remain to be solved modern research has changed the traditional concept of sleep by two essential new aspects formulated by Jouvet (71 72) 1) Our brain, like our kidneys and heart but unlike our muscular system does not rest during sleep On the contrary it undergoes active reorganization rather than a real inhibition, and so sleep seems to be an active phenomenon 2) Behavioural sleep does not proceed from a single process but is the manifestation of two different states of nervous activity

APPLIED SLEEP STUDIES IN PEDIATRICS

Continuous polygraphic recordings during sleep have become a new tool for the diagnosis and investigation of a variety of diseases in infants and children Under pathologic conditions both quantitative and qualitative changes in sleep patterns may occur revealing disturbances in the complex nervous and neurochemical feedback mechanisms responsible for the coordination and homeostasis in the sleep states

In severely damaged newborns Monod *et al* (92) found a complete lack of cyclic organisa

tion of sleep Lesser degrees of neurological abnormality will lead to abnormally frequent and irregular changes of the sleep states (85 116) Since NREM sleep is the state with the highest degree of homeostasis it is usually the first to show alterations under pathologic conditions Its relative amount may be decreased or the coordination of the recorded parameters may be disturbed This has been seen in infants with milder degrees of birth trauma (121) in infants of diabetic mothers (135) and in infants of heroin addicted mothers (134) A decrease of REM sleep time has been shown by Petre Quadens *et al* (111 112) in a somewhat inhomogenous group of mentally retarded patients The amount of single REM was found to be decreased in autistic children (96) whilst it was increased after acute head injury The precise time schedule after which sleep patterns develop provides the possibility to use sleep recordings for the determination of gestational age in infants (103) In certain metabolic disorders as for instance phenylketonuria (54) sleep studies may reveal changes in the EEG at a time routine EEG recordings still appear inconspicuous It is hoped that quantitative analysis of these changes will provide a possibility to control objectively the effect of treatment such studies are at present carried out in our laboratory by Schulte and co-workers The absence or decrease of sleep spindles during NREM sleep and their increase under hormone substitution are new criteria for the diagnosis and control of hypothyroidism (106 36) New information about the pathophysiology of certain forms of epilepsy has been gained from EEG recordings during the different sleep stages (89 129 137) Of interest for the child psychiatrist is the finding of Broughton (19) that the classic sleep disorders nocturnal enuresis somnambulism and nightmare—occur preferentially during arousal from NREM sleep and are virtually never associated with the rapid-eye movement dreaming state A wide variety of diseases causing structural or functional disturbances of the brain is awaiting further exploration by polygraphic sleep studies in addition to anatomical metabolical biochemical and psychological investigations

It is obvious that all stimulus—response studies in young infants—neurological investigations evoked response studies audiometric testing (56) or psychophysiological investigations (61)—require careful monitoring of the state of the subject

Sleep studies will provide useful information about the functional organisation of the brain during normal ontogenetic development Vice versa the pediatrician is in the favourable position that he can use developmental changes in the sleep pattern as a tool for studying sleep mechanism in man

REFERENCES

1 Akiyama Y Schulte F J Schultz M A & Parmelee A H Acoustically evoked responses in premature and full term newborn infants *Electroenceph Clin Neurophysiol 26* 371 1969

2 Arduini A Berlucchi G & Strata P Pyramidal activity during sleep and wakefulness *Arch Ital Biol 101* 530 1963

3 Aserinsky E & Hounskaecht T R Respiration associated with the REM stage of human sleep *Fed Proc 24* 339 1965

4 Aserinsky E & Kleitman N Regularly occurring periods of eye motility and concomitant phenomena during sleep *Science 118* 273 1953

5 — Two types of ocular motility occurring in sleep *J Appl Physiol 8* 1 1955

6 — A motility cycle in sleeping infants as manifested by ocular and gross bodily activity *J Appl Physiol* 8 11 1955

7 Baldissera F Broggi G & Mancia M Monosynaptic and polysynaptic spinal reflexes during physiological sleep and wakefulness *Arch Ital Biol 104* 112 1966

8 — Presynaptic inhibition of trigeminal afferent fibers during the rapid eye movements of desynchronized sleep *Experientia* 22 754 1966

9 Baldissera F Cesa Bianchi M G & Mancia M Phasic events indicating presynaptic inhibition of primary afferents to the spinal cord during desynchronized sleep *J Neurophysiol* 29 871 1966

10 Beintema D J *A neurological study of newborn infants* Heinemann London 1968

11 Berger R J Tonus of extrinsic laryngeal muscles during sleep and dreaming *Science 134* 840 1961

12 — Oculomotor control a possible function of REM sleep *Psychol Rev 76* 144 1969

13 Berlucchi G Munson J B & Rizzolatti G Changes in click evoked responses in the auditory system and the cerebellum of free moving cats during sleep and waking *Arch Ital Biol 105* 118 1967

14 Bernuth H v & Prechtl H F R Vestibulo-ocular response and its state dependency in newborn infants *Neuropadiatrie 1* 11 1969

15 Bizzi E Changes in the orthodromic and antidromic response of optic tract during the eye movements of sleep *Physiologist 8* 113 1965

16 — Discharge patterns of single geniculate neurons during the rapid eye movements of sleep *J Neurophysiol 29* 1087 1966

17 Bizzi E Pompeiano O & Somogyi I Spontaneous activity of single vestibular neurons of unrestrained cats during sleep and wakefulness *Arch Ital Biol 102* 308 1964

18 Brebbia R D & Altshuler K Z Oxygen consumption rate and electroencephalographic stage of sleep *Science 150* 1621 1965

19 Broughton R J Sleep disorders disorders of arousal? *Science 159* 1070 1968

20 Canestrini S *Über das Sinnesleben des Neugeborenen* Springer Berlin 1913

21 Carli G Diete Spiff K & Pompeiano O Inhibizione presinaptica e postsinaptica nel nucleo cuneato durante il sonno *Boll Soc Ital Biol Sper 42* 640 1966

22 Caveness W F *Atlas of electrocephalography in the developing monkey macaca mulatta* Addison Wesley Reading Mass 1962

23 Chase M H Sterman M B & Clemente C D Maturational pattern of sleep and wakefulness in the kitten *Fed Proc 24* 339 1965

24 Cooper R & Hulme H Intracranial pressure and related phenomena during sleep *J Neurol Neurosurg Psychiat 29* 564 1966

25 Delange M M Castan P Cadilhac J & Passouant P Les divers stades du sommeil chez le nouveau né et le nourrisson *Rev Neurol* (Paris) *107* 271 1962

26 Dement W The occurrence of low voltage fast EEG patterns during behavioral sleep in the cat *Electroenceph Clin Neurophysiol 10* 291 1958

27 — Eye movements during sleep *in* M B Bender (ed) *The oculomotor system* Harper & Row New York 1964

28 Dement W & Kleitman N Cyclic variations in EEG during sleep and their relations to eye movements body motility and dreaming *Electroenceph Clin Neurophysiol 9* 673 1957

29 — The relation of eye movements during sleep to dream activity An objective method for the study of dreaming *J Exp Psychol 53* 339 1965

30 Dement, W & Wolpert E The relation of eye movements body motility and external stimuli to dream content *J Exp Psychol 55* 543 1958

31 Dittrichova J Nature of sleep in young infants *J Appl Physiol 17* 543 1962

32 — Development of sleep in infancy *J Appl Physiol 21* 1243 1966

33 Dittrichova J & Paul K The course of night sleep in infants *Act Nerv Sup 8* 202 1966

34 Dreyfus Brisac C Electroencephalography in infancy *in* F Linneweh (ed) *Die physiologische Entwicklung des Kindes* Springer Berlin Gottingen and Heidelberg 1959

35 — The electrocephalogram of the premature infants and fullterm newborn Normal and abnormal development of waking and sleeping patterns *in* P Kellaway & I Petersen (eds) *Neurological and Electroencephalographic Correlative Studies in Infancy* Grune & Stratton New York 1964

36 — Sleep ontogenesis in early human prematurity from 24 to 27 weeks of conceptual age *Develop Psychobiol 1* 162 1968

37 Dreyfus Brisac C Fischgold H Samson D Saint Anne Dargassies S Ziegler T Monod N & Blanc C Veille sommeil et reactivite sensorielle chez le premature et le nouveau ne *Electroenceph Clin Neurophysiol* Suppl *6* 418 1956

38 Dreyfus Brisac C & Monod N Sleep of premature and full term neonates—a polygraphic study *Proc Roy Soc Med 58* 6 1965

39 *Dreyfus Brisac C Samson D* Blanc C & Monod N L electroencephalogramme de l enfant normal de moins de 3 ans *Etudes Neo-Natales 7 143 1958*

40 Dubois Dalcq M Contribution a l etude du fonctionnement du systeme nerveux du nouveau ne a terme *Acta Paediat Belg 21* 257 1967

41 Eliet Flescher J & Dreyfus Brisac C Le sommeil du nouveau né et du premature II Relations entre l electroencephalogramme et l electromyogramme mentonnier au cours de la maturation *Biol Neonat 10* 316 1966

42 Evarts E V Effects of sleep and waking on activity of single units in the unrestrained cat, *in* G E W Wolstenholme & M O Connor (eds) *The nature of sleep* Churchill London 1961

43 — Activity of neurons in visual cortex of the cat during sleep with low voltage fast EEG activity *J Neurophysiol 25* 812 1962

44 — Temporal patterns of discharge of pyramidal tract neurons during sleep and waking in the monkey *J Neurophysiol 27* 152 1964

45 Feinberg I & Carlson V R Sleep variables as a function of age in man *Arch Gen Psychiat 18* 239 1968

46 Fisher C Gross J & Zuch J Cycle of penile erection synchronous with dreaming (REM) sleep *Arch Gen Psychiat 12* 29 1965

47 Gabersek V & Scherrer J Etude électrophysiologique du sommeil de l enfant *Rev Neuropsychiat Infant 14* 121 1966

48 Gassel M M Ghelarducci B Marchiafava P L & Pompeiano O Phasic changes in blood pressure and heart rate during rapid eye move

ment episodes of desynchronized sleep in unrestrained cats *Arch Ital Biol* 102 530 1964
49 Gassel M M Marchiafava P L & Pompeiano O Phasic changes in muscular activity during desynchronized sleep in unrestrained cats *Arch Ital Biol* 102 449 1964
50 — Tonic and phasic inhibition of spinal reflexes during deep sleep desynchronized sleep in unrestrained cats *Arch Ital Biol* 102 471 1964
51 Gassel M M & Pompeiano O Pyramotor function during sleep in unrestrained cats *Arch Ital Biol* 103 347 1965
52 Giaquinto S Pompeiano O & Somogyi I Supraspinal modulation of heteronymous monosynaptic and of polysynaptic reflexes during natural sleep and wakefulness *Arch Ital Biol* 102 45 1964
53 Globus L & van Velzer C Innate sleep rhythms *Brain* 88 1043 1965
54 Gross H P & Schulte F J Über die veränderte Spindelaktivität im Schlaf-EEG bei Kindern mit Phenylketonurie *Z Kinderheilk* 105 374 1969
55 Gupta J M & Scopes J Observations on blood pressure in newborn infants *Arch Dis Childh* 40 637 1965
56 Hartmann E L The D-state *New Engl J Med* 273 30 and 87 1965
57 Hodes R & Dement W Depression of electrically induced reflexes ("H reflexes") in man during low voltage EEG sleep *Electroenceph Clin Neurophysiol* 17 617 1964
58 Hodes R & Gribetz I H reflexes in normal human infants depression of these electrically induced reflexes (EIRs) in sleep *Proc Soc Biol* 110 577 1962
59 Hrbek A Hrbková M & Lenard H G Somatosensory auditory and visual evoked responses in human infants during sleep and wakefulness *Electroenceph Clin Neurophysiol* 26 597 1969
60 Hutt S J Hutt C Lenard H G Bernuth H v & Muntjewerff W J Auditory responsivity in the human neonate *Nature* 218 888 1968
61 Hutt S J Lenard H G & Prechtl H F R Psychophysiological studies in newborn infants in L P Lipsitt & C C Spiker (ed) *Advances in child development and behavior* vol IV Academic Press New York 1969
62 Huttenlocher P R Evoked and spontaneous activity in single units of medial brain stem during natural sleep and waking *J Neurophysiol* 24 451 1961
63 Jacobson A Kales A Lehmann D & Hoedemaker F S Muscle tonus in human subjects during sleep and dreaming *Exp Neurol* 10 418 1964
64 Jeannerod M Mouret J & Jouvet M Etude de la motricité oculaire au cours de la phase paradoxale du sommeil chez le chat *Electroenceph Clin Neurophysiol* 18 554 1965

35 — 70 703 Acta Paediat Sca 5 59

65 Joppich G & Michaelis R Über zerebrale Aktivitäten bei Neugeborenen und jungen Säuglingen *Dtsch Med Wschr* 92 2295 1967
66 Joppich G & Schulte F J *Neurologie des Neugeborenen* Springer Berlin Heidelberg and New York 1968
67 Jouvet M Telencephalic and rhombencephalic sleep in the cat in G E W Wolstenholme & M O Connor (eds) *The nature of sleep* Churchill London 1961
68 — Recherches sur les structures nerveuses et les mécanismes responsables des différentes phases du sommeil physiologique *Arch Ital Biol* 100 125 1962
69 — Paradoxical sleep—a study of its nature and mechanisms in K Akert C Bally & J P Schadé (eds) Progress in brain research vol XVIII Elsevier Amsterdam 1965
70 — Sommeil et mouvements *Bull Schweiz Akad Med Wiss* 22 287 1966
71 — The states of sleep *Sci Amer* 216 62 1967
72 — Neurophysiology of the states of sleep *Physiol Rev* 47 117 1967
73 Kanzow E Krause D & Kühnel H Die Vasomotorik der Hirnrinde in den Phasen desynchronisierter EEG Aktivität im natürlichen Schlaf der Katze *Arch Ges Physiol* 274 593 1962
74 Kawamura H Whitmoyer D I & Sawyer C H Temperature changes in the rabbit brain during paradoxical sleep *Electroenceph Clin Neurophysiol* 21 469 1966
75 Khatri I M & Freis E D Hemodynamic changes during sleep *J Appl Physiol* 22 867 1967
76 Kleitman N *Sleep and wakefulness* University of Chicago Press Chicago 1963 2nd edition
77 Kleitman N & Engelmann T G Sleep characteristics of infants *J Appl Physiol* 6 269 1953
78 Koella W P Feldstein A & Czicman J S The effect of parachlorophenylalanine on the sleep of the cats *Electroenceph Clin Neurophysiol* 25 481 1968
79 Kohler W C Coddington D & Agnew H W Sleep patterns in 2 year-old children *J Pediat* 72 228 1968
80 Korner A F REM organization in neonates *Arch Gen Psychiat* 19 330 1968
81 Kubota K Iwamura Y & Niimi Y Monosynaptic reflex and natural sleep in the cat *J Neurophysiol* 28 125 1965
82 Kubota K & Tanaka R The fusimotor activity and natural sleep in the cat *Brain Research* 3 198 1966
83 Lenard H G The development of sleep spindles in the EEG during the first two years of life *Neuropaediatrie* 1 264 1970
84 Lenard H G Bernuth H v & Prechtl H F R Reflexes and their relationship to behavioral state in the newborn *Acta Paediat Scand* 57 177 1968
85 Lenard H G & Prechtl H F R Polygraphische Untersuchungen bei neurologisch ge

storten Neugeborenen *Mschr Kinderheilk 116* 322 1968
86 Loomis A L Harvey E N & Hobart G A Cerebral states during sleep as studied by human brain potentials *J Exp Psychol 21* 127 1937
87 Mandell A J & Mandell M P Biochemical aspects of rapid eye movement sleep *Amer J Psychiat 122* 391 1965
88 Mandell M P Mandell A J Rubin R T Brill P Rodnick J Sheff R & Chaffey B Activation of the pituitary adrenal axis during rapid eye movement sleep in man *Life Sci 5* 583 1966
89 Martinius J Über das Verhalten epileptogener Aktivität im Schlaf beim Säugling *Mschr Kinderheilk 116* 101 1968
90 Meier G W & Berger R J Developement of sleep and wakefulness patterns in the infant rhesus monkey *Exptl Neurol 12* 257 1965
91 Monod N & Pajot N Le sommeil du nouveau né et du prématuré I Analyse des études polygraphiques (mouvements oculaires respiration et EEG) chez le nouveau né à terme *Biol Neonat 8* 281 1965
92 Monod N Eliet Flescher J & Dreyfus Brisac C *Le sommeil du nouveau né et du prematuré III* Les troubles de l organisation du sommeil chez le nouveau né pathologique Analyse des etudes polygraphiques *Biol Neonat 11* 216 1967
93 Morrison A R & Pompeiano O An analysis of the supraspinal influences acting on motoneurons during sleep in the unrestrained cat *Arch Ital Biol 103* 497 1965
94 — Central depolarization of group Ia afferent fibres during desynchronized sleep *Arch Ital Biol 103* 517 1965
95 Mouret J *Les Mouvements Oculaires au cours du Sommeil Paradoxal* Thèse Imprimerie des Beaux Arts Lyon 1964
96 Ornitz E M Ritvo E R Brown M B La Franchi S Parmelee T & Walter R D The EEG and rapid eye movements during REM sleep in normal and autistic children *Electroenceph Clin Neurophysiol 26* 167 1969
97 Ornitz E M Ritvo E R Carr E M Panman L M & Walter R D The variability of the auditory averaged evoked response during sleep and dreaming in children and adults *Electroenceph Clin Neurophysiol 22* 514 1967
98 Oswald I Slow neurophysiological swings *Ann NY Acad Sci 138* 616 1967
99 Oswald I Ashcroft G W Berger R J Eccleston D Evans J I & Thacore V R Some experiments in the chemistry of normal sleep *Brit J Psychiat 112* 391 1966
100 Othmer E Hayden M P & Segelbaum R Encephalic cycles during sleep and wakefulness in humans A 24 hour pattern *Science 164* 447 1969
101 Parmelee A H Sleep patterns in infants *Acta Paediat Scand* 50 160 1961
102 Parmelee A H Akiyama Y Schultz M A Wenner W H Schulte F J & Stern E The *electroencephalogram in active and quiet sleep in infants in* P Kellaway & I Petersén (eds) *Clinical electroencephalography of children* Almqvist & Wiksell, Stockholm 1968
103 Parmelee A H Schulte F J Akiyama Y Wenner W H Schultz M A & Stern E Maturation of EEG activity during sleep in premature infants *Electroenceph Clin Neurophysiol 24* 319 1968
104 Parmelee A H Wenner W H Akiyama Y Schultz M A & Stern E Sleep states in premature infants *Develop Med Child Neurol 9* 70 1967
105 Passouant P Cadilhac J & Delange M Le sommeil du nouveau né Considerations sur la periode des mouvements oculaires *Arch Franc Pediat 22* 087 1965
106 Passouant P Passouant Fontaine T & Cadilhac J L influence de l hyperthyroidie sur le sommeil *Rev Neurol (Paris) 115* 353 1966
107 Peiper A *Die Eigenart der kindlichen Hirntaetigkeit* VEB Thieme Leipzig 1961 3rd edition
108 Petre Quadens O Etude du sommeil chez le *nouveau ne normal in Le Sommeil de Nuit Normal et Pathologique* Masson Paris 1965
109 — On the different phases of the sleep of the newborn with special reference to the activated phase or phase D *J Neurol Sci 3* 151 1966
110 — Ontogenesis of paradoxical sleep in the human newborn *J Neurol Sci 4* 153 1967
111 Petre Quadens O & Jouvet M Paradoxical phase of sleep in the mentally retarded *J Neurol Sci 3* 608 1966
112 Petre Quadens O Jouvet M Myle O Devos J Clara R & van Heule R Recherches sur le sommeil des oligophrènes et en particulier sur le modification du tonus musculaire *Acta Neurol Psychiat Belg 66* 575 1966
113 Pisano M Rosadini G Rossi G F & Zattoni J Relations between threshold of arousal and electroencephalographic patterns during sleep in man *Physiol Behav 1* 55 1966
114 Prechtl H F R Problems of behavioral studies *in the newborn infant in* C Lehrman & R Hinde (eds) *Advances in the study of behavior* vol I Academic Press New York 1965
115 — Polygraphic studies of the full term newborn II Computer analysis of recorded data *in* R MacKeith & M Bax (eds) *Studies in infancy* Heinemann London 1968
116 Prechtl H F R Akiyama Y Zinkin P & Kerr Grant D Polygraphic studies of the full term newborn I Technical aspects and qualitative analysis *in* R MacKeith & M Bax (eds) *Studies in infancy* Heinemann London 1968
117 Prechtl H F R Kerr Grant D Lenard H G & Hrbek A The lip tap reflex in the awake *and sleeping newborn infant A polygraphic* study *Exp Brain Res 3* 184 1967
118 Prechtl H F R & Lenard H G A study of

eye movements in sleeping newborn infants *Brain Research* 5 477 1967

119 — Verhaltensphysiologie des Neugeborenen in F Linneweh (ed) *Fortschritte der Pädologie* vol II Springer Berlin Heidelberg and New York 1968

120 Prechtl H F R Vlach V Lenard H G & Kerr Grant D Exteroceptive and tendon reflexes in various behavioural states of the newborn infant *Biol Neonat* 11 159 1967

121 Prechtl H F R Weinmann H & Akiyama Y Organization of physiological parameters in normal and neurologically abnormal infants *Neuropaediatrie* 1 101 1969

122 Preyer W *Die Seele des Kindes* Fernau Leipzig 188[illegible]

123 Reding G R & Fernandez C Effects of vestibular stimulation during sleep *Electroenceph Clin Neurophysiol* 24 75 1968

124 Reite M Pegram C V Stephens L M Bixler E C & Lewis O L The effect of reserpine and monoamine oxydase inhibitors on paradoxical sleep in the monkey *Psychopharmacol* 14 12 1969

125 Reivich M Isaacs G Evarts E & Kety S The effect of slow wave sleep and REM sleep on regional cerebral blood in cats *J Neurochem* 15 301 1968

126 Roffwarg H P Dement W C Muzio J N & Fisher C Dream imagery Relationship to rapid eye movements of sleep *Arch Gen Psychiat* 7 235 1962

127 Roffwarg H P Muzio J N & Dement W C Ontogenetic development of the human sleep-dream cycle *Science* 152 604 1966

128 Ross J J Agnew H W Williams R L & Webb W W Sleep patterns in preadolescent adults An EEG EOG study *Pediatrics* 42 324 1968

129 Ross J R Johnson L C & Walter R D Spike and wave discharges during stages of sleep *Arch Neurol* 14 399 1966

130 Ruckebusch J Etude polygraphique et comportementale de l'evolution post natale du sommeil physiologique chez l'agneau *Arch Ital Biol* 101 111 1963

131 Samson Dollfus D *L'EEG du prématuré jusqu'à l'age de 3 mois et du nouveau né à terme* Thèse Toulon Tarn 1955

132 Samson Dollfus D Forthomme J & Capron E EEG of the human infant during sleep and wakefulness during the first year of life in P Kellaway & I Petersén (eds) *Neurological and electroencephalographic correlative studies in infancy* Grune & Stratton New York 1964

133 Satoh T Brain temperature of the cat during sleep *Arch Ital Biol* 106 73 1968

134 Schulman C A Alterations of the sleep cycle in heroin addicted and "suspect" newborns *Neuropaediatrie* 1 89 1969

135 Schulte F J Linke U Parl U Nolte R & Jürgens U Brain and behavioural maturation in newborn infants of diabetic mothers II Sleep cycles *Neuropaediatrie* 1 36 1969

136 Schultz M A Schulte F J Akiyama Y & Parmelee A H Development of electroencephalographic sleep phenomena in hypothyroid infants *Electroenceph Clin Neurophysiol* 25 351 1968

137 Scollo-Lavizzari G & Hess R Photic stimulation during paradoxical sleep in photosensitive subjects *Neurology* 17 604 1967

138 Shimizu A & Himwich H E The ontogeny of sleep in kittens and young rabbits *Electroenceph Clin Neurophysiol* 24 307 1968

139 Siegel J & Langley T D Arousal threshold in the cat as a function of sleep phase and stimulus significance *Experientia* 21 740 1965

140 Snyder F Toward an evolutionary theory of dreaming *Amer J Psychiat* 123 121 1966

141 Snyder F Hobson J A Morrison D F & Goldfrank F Changes in respiration heart rate and systolic blood pressure in human sleep *J Appl Physiol* 19 417 1964

142 Stern E Parmelee A H Akiyama Y Schultz M A & Wenner W H Sleep cycle characteristics in infants *Pediatrics* 43 65 1969

143 Tcheng F C Y & Laroche J L Phases de sommeil et sourires spontanés *Acta Psychol (Amst)* 24 1 1965

144 Valatx J L Jouvet D & Jouvet M Evolution électroencéphalographique des différents etats de sommeil chez le chaton *Electroenceph Clin Neurophysiol* 17 218 1964

145 Weitzman E D & Kremen H Auditory evoked responses during different stages of sleep in man *Electroenceph Clin Neurophysiol* 18 65 1965

146 Weitzman E D Rapport M M McGregor P & Jacoby J Sleep patterns of the monkey and brain serotonin concentration Effect of p-chlorphenylalanine *Science* 160 1361 1968

147 Williams D H & Cartwright R D Blood pressure changes during EEG monitored sleep *Arch Gen Psychiat* 20 307 1969

148 Wolff I H Observations on newborn infants *Psychosom Med* 21 110 1959

149 — *The causes controls and organization of behavior in the neonate* Psychological Issues Vol V Monograph 17 International Universities Press New York 1966

150 Zipperling H Über eine besondere Form motorischer Reizzustände beim Neugeborenen (sog Stürpchen) *Z Kinderheilk* 5 31 1913

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CASE REPORT

A CASE OF HYPERCALCEMIA AND RENAL INSUFFICIENCY IN CHILDHOOD SARCOIDOSIS?

H T LUND I TRANSBØL and I HORNUM

From the University Clinic of Pediatrics and Medical Department A and P Rigshospitalet University Hospital Copenhagen Denmark

Sarcoidosis is an uncommon disease of child hood Till 1956 only 113 cases had been described in the pediatric literature (6) Since then few additional cases have been published (1 11 13 15) A recent review is given by Jasper & Denny (8) The disease may be encountered at any age The youngest case reported is a 2 months old infant but 75% of the pediatric cases have occurred in the preadolescent or adolescent age groups (6 14)

Sarcoidosis presents symptoms from several organs and tissues the most frequent involve ment being that of the lungs and the lymphatic system The occasional occurrence of hyper calcemia is due to increased intestinal absorption of calcium which is reversed by cortisone (2 3) Increased sensitivity to vitamin D and even *to sunlight are typical features of this* type of hypercalcemia (3 16) The hyper calcemia of sarcoidosis is characterized by a rapidly progressing renal insufficiency due to formation of metastatic calcifications and by excessive hypercalcuria During remission of sarcoidosis as hyperabsorption of calcium ceases *the* hypercalcemia and hypercalcuria may be maintained for months by mobilization of metastatic calcifications (7 17)

To our knowledge only 3 cases of hyper calcemic sarcoidosis in childhood have been reported (4 15) The following case report describes a patient in whom symptoms of *hypercalcemia and renal insufficiency* were the only clinical manifestations

CASE REPORT

H B is a 13 year old girl whose father and two elder sibs are healthy At the age of 18 her mother had a renal stone removed Pregnancy birth and *postnatal development were not contributary* The patient s intake of vitamin D had always been within normal limits

Since the age of 12 she suffered from incessant thirst polyuria bad appetite fatigue and loss of weight Five months later she was admitted to a hospital where she was found to be suffering from *renal insufficiency* Urinary sediment and cultures and X ray examination of the chest and urography were normal

On admission at the pediatric department after year her general health was good She looked pale but otherwise the physical examination was completely normal

Clinical and laboratory studies

Examination of the blood revealed a moderate normochromic anemia and a leucocyte count of 3600/μl with a normal differential distribution Thrombo*cyte count and clotting time were normal* SR 40 mm hour Serum protein 7 8 g/100 ml gamma globulin 1 4 g 100 ml (normal range = 0 6–1 10 g 100 ml) other globulins and serum albumin were normal Rheumatoid arthritis test antistreptolysin and antistreptohyaluronidase titers were *normal L E* cell phenomenon was negative Two months after BCG vaccination Mantoux I (0 02 μg tuberculin 0 1 ml) and II (0 2 μg tuberculin/0 1 ml) tests were negative Serum bilirubin transaminase alkaline

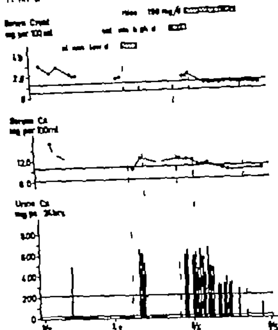


Fig. 1. Serum creatinine, serum calcium and urinary excretion of calcium during admission. Calcium low diet: 200 mg calcium and 270 mg phosphorus per day. Calcium high diet: 870 mg calcium and 1170 mg phosphorus per day. Cortisone 150 mg/d per 70 kg body weight (see text).

phosphatases and serum phosphorus concentrations were normal. Serum calcium values were elevated (Fig. 1).

Renal function as measured by the endogenous clearance of creatinine and serum creatinine was decreased (Fig. 1 and Table 1). Blood pressure 120/80 mm Hg. Urinary volumes were high, averaging 3000 ml/24 hour with a specific gravity below 1.010. Urinary sediment and cultures were normal. Examination for sugar and protein negative.

X rays of the skeleton, teeth, lungs, oesophagus, trachea and urinary system disclosed nothing abnormal.

Eye examination (split lamp) revealed typical band keratitis.

An open *renal biopsy* was performed which showed nephrocalcinosis (Fig. 2) but no granulomas.

Further studies of calcium and phosphorus metabolism. Clinical course

On a diet containing 200 mg calcium and 270 mg phosphorus the 24-hourly urinary excretion of phosphorus was 832, 825 and 806 mg respectively. The urinary excretion of calcium was greatly increased (Fig. 1). Determinations of the phosphate excretion index (PEI) a.m. Nordin & Fraser (12) amounted to +0.4...+0.33 (normal range = −0.09–+0.09) (Table 1). Serum calcium fractions and the tubular reabsorption of calcium (TRCa%) were measured on a standard diet a.m. Trumbull *et al.* (18) (Table 1). The finding of hypercalcemia with a very low TRCa% 87.9% was typical of a non parathyroid hypercalcemia (Fig. 3).

Cortisone test a.m. Dent (5) by use of 100 mg of cortisone per day (150 mg per 70 kg body weight) led to normalization of serum calcium within 10 days. Also the urinary calcium excretion and serum creatinine were gradually normalized (Fig. 1 and Table 1) and urinary volumes and specific gravity became normal. Concomitantly the clinical symptoms disappeared.

A paratracheal lymphnode biopsy, a bone marrow

Table 1. *Serum calcium fractions, serum phosphate and the renal handling of calcium and phosphate before and during administration of cortisone*

	Serum				Urine[b]				
	TOCa (mg/100 ml)	UFCa	Ca	P	CCr (ml/min)	Ca (mg/24 h)	P	TRCa (%)	24-h PEI
Normal range	9–10.6	6.55–7.65	6.00–6.60	2.5–4.6	82–105[d]	26–224[d]			−0.08–+0.09
The last two days before cortisone	12.1 12.5	9.30 9.10	8.05 7.90	3.0 3.4	34 40	589 590	840 860	87.2 88.6	+0.42 0.33
Cortisone									
Day 11–12	10.3	7.90	6.75	2.8	66	365	1011	95.1	+0.30
Day 13–14	10.1	7.70	7.15	2.6	71	335	992	95.7	+0.30

TOCa = total calcium, UFCa = ultrafiltrable calcium, Ca = ionized calcium.
[b] TRCa = the tubular reabsorption of calcium expressed as per cent of the filtered load of calcium.
24-h P.E.I. = the 24-hour phosphate excretion index. CCr = the 24-hour endogenous clearance of creatinine.
In adult subjects (n = 83).

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Fig. 4. Biopsy from striated muscle (femur). A multinucleated giant cell is seen surrounded by round cells (250×1).

excretion tests offer no such distinction in states of hypercalcemia, especially not when renal insufficiency supervenes (18, 19).

The exclusion of hyperparathyroidism prompted the performance of additional biopsies. The changes seen in the muscle biopsy were not pathognomonic of sarcoidosis as no granulomas were found. Nevertheless the sum of pathologic findings comprising histological changes, negative Mantoux tests in spite of previous BCG vaccination, increase of the gamma globulin fraction, elevated sedimentation rate, and of course the hypercalcemia shows a pattern suggesting sarcoidosis as the only possible diagnosis.

This case report demonstrates that hypercalcemia should be suspected in *all* cases of renal insufficiency irrespective of the X ray findings. In addition sarcoidosis should be excluded as a possible cause of unexplained hypercalcemia even in the absence of other clinical evidence of this disease. Unnecessary



Fig 2 Renal biopsy showing focal calcifications but no granulomatous lesions Some of the tubules are dilated The interstitial tissue is infiltrated with chronic inflammatory cells

aspirate and a skin biopsy were negative A striated muscle biopsy from the femur showed perivascular inflammatory changes and multinucleated giant cells No granulomas were found (Fig 4)

On treatment with prednisone 10 mg per day and a diet low in calcium and vitamin D the patient was discharged in good health for continued out patient treatment At control admission 2 months later she was without symptoms Renal function serum calcium and urinary calcium excretion were normal

DISCUSSION

The reported patient presents none of the classical signs or symptoms of sarcoidosis Her complaints can all be ascribed to the hypercalcemia and the accompanying renal insufficiency Metastatic calcifications only demonstrable by slit lamp examination and renal biopsy are very frequent but not pathognomonic findings in states of non parathyroid hypercalcemia (sarcoidosis vitamin D intoxication milkalkali syndrome etc) These calcifications are to a remarkable degree reversible Despite hypercalcemic symptoms for more than a year correction of the hypercalcemia is followed by a rapid normalization of renal function (7 17) For a child the degree of hypercalcuria is remarkable It exceeds the levels found in a material of 57 adult cases of hyperparathyroidism (19) Also this is an important point in the differential diagnosis as in hyperparathyroidism urinary calcium excretion is generally only moderately increased or even normal in contrast to the extensive hypercalcuria seen in many cases of hypercalcemic sarcoidosis

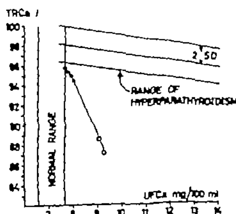


Fig 3 The tubular reabsorption of calcium (TRCa) related to the concentration of ultrafiltrable calcium in serum (UFCa) in the patient *before* (O) and *during* (●) administration of cortisone The range of hyperparathyroidism has been published previously (17)

In ruling out hyperparathyroidism the measurement of the TRCa* was valuable as was the cortisone test The former proved to be clearly below the range of hyperparathyroidism (Fig 3) Determination of TRCa is useful in the differential diagnosis of hypercalcemia *irrespective* of the degree of renal insufficiency at least at clearances of creatinine ranging from 13 to 177 ml/min (18 19) The phosphate



Fig. 4. Biopsy from striated muscle (femur). A multinucleated *giant cell is seen surrounded by* round cells (250 × 1).

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neck explorations have been carried out in some twenty adult patients with hypercalcemic sarcoidosis of which seven had normal X rays of the chest (20)

SUMMARY

In a 13 year-old girl an unusual case of hyper calcemia is reported Symptoms of hyper calcemia and renal insufficiency were the only complaints Physical examination, and X rays were all negative Investigation of calcium metabolism revealed hypercalcemia excessive hy percalcuria and discrete metastatic calcifica tions, discovered by slit lamp examination and renal biopsy The tubular reabsorption of cal cium (TRCa%) and the cortisone test both indicated a non parathyroid origin of hyper calcemia Negative Mantoux tests in spite of previous BCG vaccination increase of the serum gamma globulin fraction elevated sedi mentation rate and a muscle biopsy showing chronic inflammatory changes and giant cells were the positive findings which rendered sar coidosis the only probable diagnosis although no typical granulomas were found Finally treatment with prednisone normalized the cal cium metabolic disturbances and renal func tion became normal

REFERENCES

1 Andersen H A Sarcoidosis (Boeck) in a child treated with cortisone and ACTH *Acta Paediat Scand 45* 343 1956
2 Anderson J Dent C E Harper C & Philpot G R Effect of cortisone on calcium metabolism in sarcoidosis with hypercalcemia Possible ac tions of cortisone and vitamin D *Lancet II* 720 1954
3 Bell N H Gill J R Jr & Bartter F C On the abnormal calcium absorption in sar coidosis Evidence for increased sensitivity to vitamin D *Amer J Med 36* 500 1964
4 van Creveld S Disturbances of the metabolism in Bernier Boeck s disease *Ann Paediat* (Basel) *157* 1 1941
5 Dent C E Cortisone test for hyperparathyroid ism *Lancet I 230 1956*
6 McGovern J P & Merrit D H Sarcoidosis in childhood *Advanc Pediat* 8 97 1956
7 Hornum I Transbøl I & Hahnemann S Metabolic balance and calcium 47 data in hy percalcaemic sarcoidosis with and without hyper parathyroidism in J Turiaf & J Chabot (eds) *La Sarcoidose* Rapp IV conf internat Paris 1966 Masson & C édit Paris 1967 p 610
8 Jasper P L & Denny F W Sarcoidosis in children *J Pediat 73* 499 1968
9 Kogut M D & Neumann L L Renal in volvement in Boeck s sarcoidosis *Pediatrics 28* 410 1961
10 Knapp E L Factors influencing the urinary excretion of calcium in normal persons *J Clin Invest 26 182 1947*
11 Mandi L Thoracic sarcoidosis in childhood *Acta tuberc scand 45* 256 1964
12 Nordin B E C & Fraser R Assessment of urinary phosphate excretion *Lancet I* 947 1960
13 Scadding J G *Sarcoidosis* Eyre & Spottiswood London 1967
14 Schmid D Die klinischen Erscheinungsformen der epithelzelligen Granulomatose im Kinder alter *Mschr Kinderheilk 98* 489 1950
15 Siltzbach L E & Greenberg G M Childhood sarcoidosis—a study of 18 patients *New Eng J Med 279* 1239 1968
16 Taylor R L Lynch H J Jr & Wysor W G Jr Seasonal influence of sunlight on the hyper calcemia of sarcoidosis *Amer J Med 34* 221 1963
17 Transbøl I Hahnemann S & Hornum I The tubular reabsorption of calcium in the differ ential diagnosis between hypercalcemic sarcoido sis and primary hyperparathyroidism in J Tu riaf & J Chabot (eds) *La Sarcoidose* Rapp IV conf internat Paris 1966 Masson & C édit Paris 1967 pp 616 and 645
18 — The tubular reabsorption of calcium in primary hyperparathyroidism and in non para thyroid hypercalcemia *Acta Med Scand 184* 31 1968
19 Transbøl I Hornum I Hahnemann S Has ner E Øhlenschlæger H Diemer H & Lock wood K The tubular reabsorption of calcium in the differential diagnosis of hypercalcemia Further experience *Acta Med Scand* To be pub lished
20 Winnacker J L Becker K L & Katz S Endocrine aspects of sarcoidosis *New Eng J Med 278* 427 1968

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CASE REPORT

CONGENITAL ATRIAL FLUTTER

Report of a Case

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The term congenital atrial flutter has been used to indicate that the flutter is believed to have been present before birth and has been documented in the immediate neonatal period (4) In the literature up to 1963 Caddell & Whittemore (4) had found 10 cases which met the criteria for congenital atrial flutter We have found in the literature another 6 case reports meeting these criteria (3–5 7 8)

This report of a case illustrates the clinical significance etiological problem and therapeutic implications of this arrhythmia

CASE REPORT

The patient was the first child of a healthy mother member of the hospital staff The mother had had measles and parotitis in her childhood During the pregnancy she had had phlebitis in the leg In the 4th to 28th weeks of pregnancy she had taken care of an aged patient with manifest parotitis In the 34th week of gestation the foetal heart sounds were found to be rapid up to 200 per minute Owing to continuing foetal tachycardia the labour was induced 4 days after the calculated term Foetal tachycardia continued through the delivery at the rate of 130 to 00 per minute

A girl was born on Nov 11 1967 in good condition with a birth weight of 3 600 g and an Apgar score of 9 In the local maternity hospital no special attention was subsequently devoted to the child She was discharged at the age of 9 days in good condition

For the first month the girl was well Her gain in weight during this time was 450 g In the 5th week she became tired fed poorly coughed and vomited and was admitted to the Department of Pediatrics University of Oulu The girl s general condition had deteriorated Cyanosis was noted around the mouth and her respiration was slightly laboured The pulse rate was rapid 160 per minute and irregular A second-degree systolic murmur was heard on auscultation of the heart Radiography revealed an enlarged heart volume 410 ml/m The electrocardiogram (Fig 1 A) showed atrial flutter the atrial rate was 420 and ventricular rate 136 per minute Deslanoside 0 02 mg/kg was given intramuscularly over a 24-hour period followed by a daily maintenance dose of 0 01 mg/kg for 5 days and continued with oral digoxin 0 02 mg/kg The girl lost 300 g of weight in a fortnight but recovered

She continued to be in good condition until at the age of 4 months she became restless and began to vomit after which she was readmitted Her general condition had slightly deteriorated No oedema was present. The heart rate was somewhat irregular 160 per minute A fourth degree systolic murmur was heard from the heart with punctum maximum in the left fourth intercostal space The liver was palpable 3 cm below the costal margin. Radiography revealed an enlarged heart of 650 ml/m the right atrium and right ventricle were dilated The pulmonary vascular markings were normal The electrocardiogram showed an atrial flutter the atrial rate was 360 and ventricular rate 160 per minute The treatment was increased with 0 5 mg/kg of furosemide The child s condition improved but tachycardia continued

The child was discharged but had to be re admitted at the age of 4 / months for difficult respiration and oedema Oedema was pronounced in the legs the liver was palpable 6 cm below the costal margin

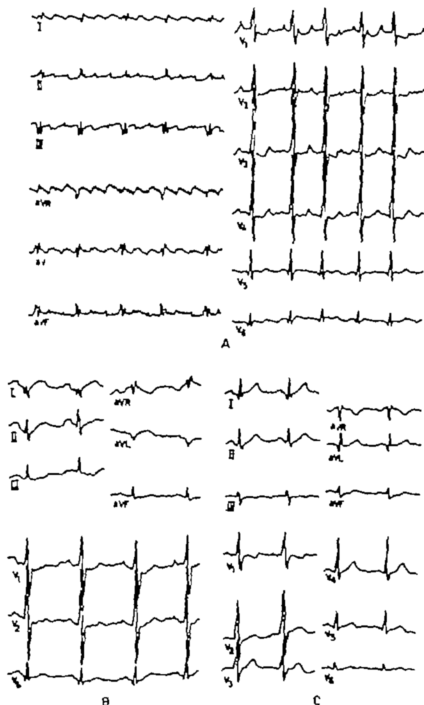


Fig 1 (A) Electrocardiogram at of 5 weeks showing flutter w atrial rate of 470 per minute an tricular rate of 165 per minute ((C) Electrocardiograms at the 5 and 11 months showing th rhythm

and a fifth degree systolic murmur was heard from the heart Radiography showed a further enlargement of the heart and a slightly increased pulmonary vascularity The ECG still revealed an atrial flutter atrial rate of 450 and ventricular rate of 180 per minute In addition to digitalis (dose 0 02 mg/kg) the girl was given furosemide (dose 7 mg/kg) for 4 days She lost 400 g of weight in 8 days and seemed better Treatment was continued at home with digoxin (dose 0 02 mg/kg) and furosemide (dose 2 5 mg/kg)

Subsequently the girl has been well Examination at the age of 5 ¹/₂ months showed no abnormal features Her general condition was good pulse rate 106 per minute and completely regular A fourth degree systolic murmur was heard from the The liver was palpable 1 / cm below the margin The ECG (Fig 1 B) revealed a sinus rh rate 110 per minute P II was 0 35 mV du 0 50 sec The dosage of furosemide and di was now gradually reduced until at the age months they were discontinued The girl was completely symptom free in good condition without any murmurs on auscultation of the Radiography still showed a large heart volume ml/m The ECG (Fig 1 C) showed sinus rhy rate 115 per minute The P wave in II lead 0 25 mV duration 0 45 sec

At the age of 13 months the girl was still sy tom free The finding on auscultation was ph

logical and the ECG still showed the sinus rhythm Radiography revealed that the heart had diminished and its volume was 430 ml/m

The viral isolation from throat and anal swab specimens at the age of 5 weeks was negative The virus antibody determinations at the age of 6 and 8 weeks disclosed no significant complement fixation (CF) antibody titres against cytomegalo adeno influenza A and B parainfluenza 1 and 2 or respiratory syncytial viruses The CF antibody titre against the parotitis virus at the age of 8 weeks was 1 8 The skin test with mumps antigen at the age of 6 weeks was negative Serum immunoglobulin at the age of 2 months gave the following result IgG 400 IgA 88 and IgM 100 mg/100 ml Of these the IgA was high and IgM was slightly above the normal value for Finnish children at this age (6)

The virus antibody determinations carried out on the mother 6 weeks post partum showed a cytomegalo and parotitis antibody titre of 1 64 and an adeno and parainfluenza 2 titre of 1 32 The influenza A and B parainfluenza 1 and 2 rubeola coxsackie A B and measles titres were normal Six weeks later the cytomegalo antibody titre was 1 32 and parotitis antibody titre 1 128

DISCUSSION

The present case meets the criteria for atrial flutter Foetal tachycardia was noticed 4 weeks antenatally and atrial flutter was recorded electrocardiographically at the age of 5 weeks

The etiology of congenital atrial flutter is in most cases unknown (3 4) It may be associated with a congenital heart defect (4) or a subendocardial fibroelastosis (9)

The contact with parotitis during pregnancy recorded by McDonagh in his 2 cases of congenital atrial flutter is very interesting (8) In our case too the mother had been in contact with parotitis in the 24th to 28th week of gestation The high maternal CF antibody titre of mumps suggest a history of recent parotitis This titre aroused suspicion although it was not definitely elevated

Parotitis is apparently one of the etiological causes of fibroelastosis and in these patients a skin test with mumps antigen is often positive (12) A skin test in our case at the age of 6 weeks was negative The only slightly increased IgM quantity (6) at the age of 10 weeks contradicts the assumption of intrauterine infection (1) On the basis of these findings no etiological causal connection can be critically demonstrated between the maternal parotitis and congenital atrial flutter although in our case it was highly possible A detailed record of the history of pregnancy as regards parotitis is necessary for all patients with congenital atrial flutter in order to throw light on the etiology of this condition

Digitalis is the drug of choice for the treatment of congenital atrial flutter The sinus rhythm returns in 75% of the cases after 1 day to 4 months of treatment (5) most frequently within the first week (2 3 8 10 11) Cardioversion to normal sinus rhythm with D C defibrillator was performed in a case reported by *Hassendruck et al* (5) without significant side effects The method might be useful if sinus rhythm is not achieved within a week of digitalis therapy

SUMMARY

A case of atrial flutter which was most likely congenital is described During pregnancy the mother had been in contact with mumps A normal IgM quantity and negative skin test with a mumps antigen were however against the assumption of intrauterine infection and fibroelastosis The part possibly played by parotitis is discussed A normal sinus rhythm was achieved by therapy with digitalis and furosemide at the age of 5 months The child has at the age of 5 1/ months no signs of a congenital heart defect

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REFERENCES

1 Alford C A Schaefer J Blankenship W J Straumfjord J V & Cassady G Correlative immunologic microbiologic and clinical approach to diagnosis of acute and chronic infections in newborn infants *New Eng J Med* 277 437 1967

2 Anderson R C & Adams F H Congenital paroxysmal tachycardia *J Pediat 43* 668 1953
3 Blumenthal S Jacobs J C Steer C M & Williamson S W Congenital atrial flutter report of a case documented by intra uterine electrocardiogram *Pediatrics 41* 659 1968
4 Caddell J L & Whittemore R Pulmonary atresia with dilated right ventricle A case with congenital atrial flutter *Amer J Cardiol 12* 254 1963
5 Hausenruck A Chojnacki B & Barker H J Cardioversion of auricular flutter in a newborn infant *Amer J Cardiol 15* 726 1965
6 Immonen P Levels of the serum immunoglobulins gamma A gamma G and gamma M in the malabsorption syndrome in children *Ann Paediat Fenn 13* 115 1967
7 Landtman B & Kaasila E Auricular flutter in infancy *Acta Paediat Scand 44* 272 1955
8 McDonagh B J Congenital atrial flutter *Arch Dis Child 43* 731 1968
9 Siderides L E Antonius N A & Richlan A Unusual auricular flutter in newborn infant *J Pediat 51* 435 1957
10 Stadler H E Prenatal auricular flutter *J Pediat 33* 624 1948
11 Tarnower H & Lattin B Tachycardia in the newborn *New York J Med 42* 805 1942
12 Vosburgh J B Diehl, A M Liu C Lauer R M & Fabiyi A Relationship of mumps to endocardial fibroelastosis complement fixation hemagglutination inhibition and intradermal skin tests for mumps in children with and without endocardial fibroelastosis *Amer J Dis Child 109* 69 1965

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CASE REPORT

MYELOPROLIFERATIVE DISEASE IN A CHILD WITH MONOSOMIA OF A C GROUP CHROMOSOME

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From the Department of Paediatrics Faculty of Medicine Hradec Králové Czechoslovakia

An infant was admitted to this hospital with a leukaemoid reaction of an obscure cause in April 1963 It was followed up and several alterations were observed during nearly 4 years of duration of the disease We believe that the course of the disease and some findings are interesting and therefore we are presenting this case

CASE REPORT

L. H a girl was born on December 21 1962 to healthy parents who later gave birth to a normal male child A disease resembling the reported one had not been observed in the family The patient was admitted for the first time at the age of 4 months (on April 16 1963) because of bronchopneumonia Physical examination revealed a 5 cm enlargement of the spleen and the liver 3 cm below the costal margin The lymph nodes were not enlarged The blood count showed a leukaemoid reaction See Table 1 Bone marrow examination showed a granulocytic hyperplasia without other pathological findings The child was treated with antibiotics and prednisone The pneumonia cleared and the leukocyte count decreased to 19 500/mm³ within 3 weeks The number of nucleated red cells and immature granulocytes in the peripheral blood decreased and the hepatosplenomegaly diminished somewhat The child was discharged in good condition after 4 weeks. Other findings during the first hospitalization were as follows Bleeding time coagulation time total serum protein paper protein electrophoresis urinanalyses serum alkaline phosphatase serum glutamic oxalacetate and glutamate pyruvate transaminases, serum lactic and malic dehydrogenases were within normal limits The Paul Bunnell reaction was negative There were no radiological changes in the bones.

After discharge the child was followed as an out patient During the remaining months of the year 1963 the hepatosplenomegaly diminished slowly and the leukocyte count decreased The good general condition of the child was not influenced by the blood disorder the way of life was not restrained and no drugs were given to treat blood disease up to the age of 4 years The growth the motor and mental development were fully normal till that age Nevertheless the child was admitted to hospital three times in the years 1964 and 1965 At the age of 18 months (June 1964) a palsy of the facial nerve occurred which disappeared completely without subsequent sequelae The cytogenetic examination of the cultured bone marrow was carried out during hospitalization in December 1964 at the age of 2 years The findings are given separately At that age the spleen and the liver were palpable at the costal margin the white blood count being 5 400/mm³

In February 1965 new alterations of some mononuclears were noted These cells resembled atypical reticulum cells in leukaemic reticulosis They were observed later too but not constantly and in general their number decreased At the age of 2 1/2 years (July 1965) the girl was admitted with acute diarrhoea vomiting acetonaemia and haematuria She was treated with antibiotics and parenteral infusions of 5% glucose and salt solutions Haematuria ceased and never returned and intravenous pyelography was within normal limits The white blood count was 8 000/mm³ The temperature sedimentation rate of erythrocytes serum glutamic oxalacetate and glutamate pyruvate transaminases were normal Neither the liver nor the spleen were palpable The clinical remission was then complete

In July 1966 (3 1/2 years) the myelocytes and the normoblasts in peripheral blood were again noted but neither the spleen nor the liver was palpable The general condition of the patient was still good. In November a rash appeared consisting of small reddish it long pimples on the skin of the trunk

Table 1 *Peripheral blood counts*

Date	Age: Years	Age: Months	Erythrocytes (millions)	Reticulocytes ( )	Leukocytes*	Differential count: Myeloblasts	Promyelocytes	Myelocytes	Metamyelocytes	Neutrophils band	Neutrophils segmented	Eosinophils	Basophils	Monocytes	Lymphocytes	Lymphocytes atypical	Reticulum cells	Nucleated red cells per 100 WBC
17 4 1963	0	3	3 06	2 0	74 200	—	1	5	8	10	15	2	—	6	37	16	—	5
6 5 1963	0	4	3 50		19 500	—	—	3	3	7	12	11	—	3	29	32	—	1
1 7 1963	0	6	3 38	2 2	24 100	—	—	2	4	8	24	10	—	—	30	22	—	2
13 11 1963	0	10	3 60		15 700	—	—	—	2	6	15	6	—	3	43	25	—	1
15 5 1964	1	4	3 80		6 700	—	—	—	3	3	28	3	—	6	38	18	—	2
15 12 1964	1	11	3 89	0 9	5 400	—	—	—	1	4	28	1	—	18	30	18	—	—
2 2 1965	2	1	3 80		7 100	—	—	—	1	4	14	—	—	29	37	15	—	—
10 7 1965	2	6	3 80		8 000	—	—	—	1	4	21	1	—	1	35	17	—	—
10 2 1966	3	1	3 50		4 700	—	—	—	—	4	33	2	—	4	43	13	—	—
26 7 1966	3	7	3 21	3 6	4 200	—	—	2	2	5	29	2	—	11	46	3	—	2
21 12 1966	4	0	3 24		4 800	5	—	1	3	4	29	3	5	3	27	20	—	8
4 1 1967	4	0	2 69		4 400	2	—	1	2	3	19	5	6	3	43	6	—	55
10 1 1967	4	0	2 11	4 6	12 500	1	—	—	1	7	39	1	2	6	27	16	—	109
18 1 1967	4	0	2 94		18 300	—	—	1	1	2	37	2	2	4	35	16	—	224
23 1 1967	4	1	2 82	2 8	2 400	—	—	—	—	5	68	—	—	1	19	7	—	[illegible]
4 2 1967	4	1	1 83		700	—	—	—	1	1	18	2	—	2	59	7	10	8

* Not corrected for nucleated red cells

and of the extremities After treatment with salicyl ointment containing dexamethasone the rash disappeared and was not seen again On December 21 1966 at the age of 4 years the liver and the spleen were palpable again 3 and 4 cm below the costal margin respectively There was also noticed a mild enlargement of the cervical axillary and inguinal lymph nodes

In the first week of January 1967 the number of erythrocytes fell to 2 5 millions At the same time the number of normoblasts in the peripheral blood attained a higher level but there were no signs of significantly increased haemolysis Tonsillitis with fever appeared and the general condition of the patient deteriorated On January 10 1967 the child was admitted to hospital for the last time The number of nucleated red cells rose strikingly in the peripheral blood to a maximum 224 per *100 WBC* on January 18 Marked erythroid hyperplasia was noted in the bone marrow 88 cells being of the red series The child was treated with antibiotics transfusions prednisone and merkaptopurine The temperature returned to normal and the number of erythrocytes rose However the improvement was only temporary Towards the end of January the number of all the blood cells began to decrease rapidly A bone marrow puncture performed on February 10 showed pronounced hypocellularity The temperature rose again stomatitis and a bleeding tendency appeared The parents of the patient refused further hospital care The child was discharged and died on February 20 1967 at her home at the age of 4 years and 2 months Autopsy was not allowed by the parents

Chromosome analyses

Bone marrow for cytogenetic examination was sampled on two occasions on December 17 1964 and on January 16 1967 The bone marrow cells were examined after 24–48 hours in culture without the use of phytohaemagglutinin The leukocytes of the peripheral blood were cultured three times with the addition of phytohaemagglutinin The cells from the first bone marrow specimen were characterized by monosomy for a C group chromosome Cytogenetic studies of bone marrow cells in January 1967 revealed 45 chromosomes with one member of the C group missing In addition a ring chromosome replaced a member of group C (Fig 1) No numerical or morphological chromosome abnormalities were found in the peripheral blood cells Table 2 gives the results of these examinations Buccal smears were positive for sex chromatin Analysis of the drumstic appendages of 500 segmented neutrophils revealed 15 cells with drumsticks

DISCUSSION

The above described case has been exceptional in our practice owing to its picture and diagnosis of myeloproliferative disease If we consider the myeloproliferative disease as not being a primary disease but only a reaction of the haemopoietic tissues to a primary condition we are lacking the primary diagnosis The peripheral blood counts and the bone marrow



Fig. 1. Metaphase obtained from the bone marrow preparation performed on January 16, 1967 and karyotype of the same cell showing 45 chromosomes with a ring chromosome of group C (with an arrow). One C group chromosome is missing.

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Date	Years	Months Age	Erythrocytes (millions)	Reticulocytes ( )	Leukocytes*	Differential count: Myeloblasts	Promyelocytes	Myelocytes	Metamyelocytes	Neutrophils band	Neutrophils segmented	Eosinophils	Basophils	Monocytes	Lymphocytes	Lymphocytes atypical	Reticulum cells	Nucleated red cells per 100 WBC	Thrombo-
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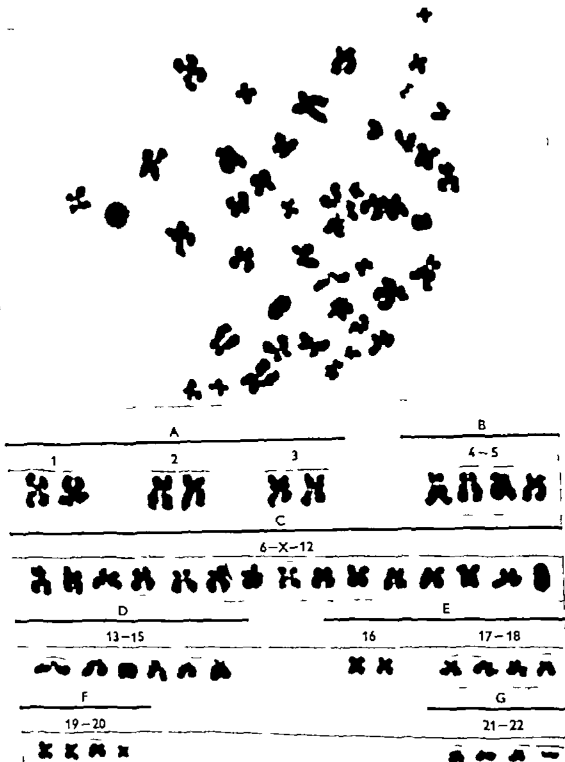


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Table 2 *Chromosomal findings*

Date	Type of tissue	Chromosome number 44	45	46	47	P	Total	Number of karyotypes Abnormal	Normal	Total	Karyotype interpretation
15 12 1964	Marrow	—	13	1	—	—	14	7	0	7	C monosomy
2 2 1965	Blood	1	3	30	—	—	34	0	3	3	Normal
8 7 1965	Blood	—	—	49	—	1	50	0	7	7	Normal
16 1 1967	Blood	—	1	9	—	—	10	0	1	1	Normal
16 1 1967	Marrow	1	23	1	—	2	27	12	0	12	C monosomy Ring chromosome of group C

findings partly the cytogenetic findings and the age exclude chronic myeloid leukaemia, chronic lymphocytic leukaemia and myeloma However there may be a relation to acute erythraemia signs of which were observed in the preterminal phase of the disease Polycythaemia vera may also be considered as a process potentially leading to myeloproliferative disease but in the observed case neither erythrocytosis nor thrombocytosis were noted in any phase of the disease

The relatively high number of lymphocytes and the presence of atypical lymphocytes during the leukaemoid reaction and later on proves that the pathological process has influenced not only the production of myeloid and erythroid cells but also the production of lymphocytes The abnormalities of mononuclear cells in the relatively benign phase of the condition led us to consider the possibility of *primary reticuloendotheliosis* However this was not proved by the later course of the disease

The disease was recognized during pneumonia and it is difficult to exclude the possibility of the leukaemoid reaction being initiated by an infection However as it occurred at the age of 4 months, it is probable that the pathological condition was inborn Although the early course of the disease was milder and anaemia and thrombocytopenia have occurred as late as in its terminal phases, the condition has a few features in common with cases described by Randall *et al* In their report no morphological or numerical abnormality in chromosome studies was found but the karyotype of bone marrow cells was analyzed in one patient only

Chromosome findings in the myeloproliferative syndrome are not uniform There have been reported (*a*) numerical changes trisomy or monosomy most frequently in the C group (*b*) structural changes deletions or breaks of the arms of a chromosome, asymmetry between the two members of the same chromosomal pair (*c*) normal findings (1 3 4, 7 9) The majority of chromosomal changes were found in direct bone marrow preparations Peripheral blood cultures without the addition of phytohaemagglutinin showed also chromosomal abnormalities (1) rarely was seen normal bone marrow with pathological karyotypes in the peripheral blood (4) Speed & Lawler (8) found the same chromosomal content of the bone marrow cells on direct examination or after short term culture during the chronic phase of chronic granulocytic leukaemia

The loss of a C group chromosome in the bone marrow cells of our patient agrees with a diagnosis of a myeloproliferative disorder It is probable that the missing C group chromosome is an autosome however no bone marrow cultures with ^{3}H thymidine have been done The chromosomal findings in this patient suggest that the abnormality appears at an early stage of the disease and may persist during the course of the illness A ring chromosome was found in the second bone marrow specimen at the stage of acute erythraemia At that time the bone marrow was dominated by erythrocytic praecursors and this finding suggests that the ring chromosome was present in

the erythrocytic praecursors Ring chromosomes has been found in acute erythroleukaemia (2)

Abnormal forms of lymphocytes were found during the disease but we have not seen any chromosomal abnormalities in the peripheral blood cultures after incubation with phytohaemagglutinin Except for short prednisone therapy in 1964 and except for the ointment with dexamethasone in autumn 1966 the patient has received no treatment with corticoids or cytostatics up to the last chromosome analysis

SUMMARY

A blood disease was recognized in a 4 months old infant It appeared first as a myeloid leukaemoid reaction with occurrence of nucleated red cells and atypical lymphocytes in peripheral blood The disease was benign at its first stages and did not influence the normal growth and development of the patient up to the age of 4 years Then an acute deterioration appeared with a picture of acute erythraemia and the child died with signs of panmyelophthisis The cytogenetic examination showed monosomia of a C group chromosome in mitoses of bone marrow cells at the age of 2 years and in addition a ring chromosome in this group in the terminal phase

REFERENCES

1 Jackson J F & Higgins L C Group C monosomy in myelofibrosis with myeloid metaplasia *Arch Intern Med 119* 403 1967
2 Krogh Jensen, M Chromosomal findings in two cases of acute erythro-leukaemia *Acta Med Scand 180* 205 1966
3 Kiossoglou K A Mitus W J & Dameshek W Cytogenetic studies in the chronic myeloproliferative syndrome *Blood 28* 241 1966
4 Padeh, B Banu G, Schatz R & Akstein E Cytogenetic studies in proliferative disorders. *Israel J Med Sci 1* 759 1965
5 Randall D L, Reiquam C W Githens J H & Robinson A Familial myeloproliferative disease *Amer J Dis Child 110* 479 1965
6 Rowley J D Blaisdell R K & Jacobson L O Chromosome studies in preleukemia. *Blood 27* 782 1966
7 Sandberg A A Ishihara, T, Crosswhite L H & Hauschka, T S Comparison of chromosome constitution in chronic myelocytic leukemia and other myeloproliferative disorders *Blood 20* 393 1962
8 Speed, D E & Lawler S D Chronic granulocytic leukaemia. The chromosomes and the disease *Lancet 1* 403 1964
9 Winkelstein A Sparkes R S & Craddock, C G Trisomy of group C in a myeloproliferative disorder *Blood 27* 722 1966

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

THE SCANDINAVIAN SOCIETY FOR PERINATAL MEDICINE

Meeting in Helsinki Jan 30 1970

Anders Rane Sumner Yaffe & Sten Orrenius (Stockholm Sweden) *Perinatal development of the hepatic microsomal drug metabolizing system*

The overall activity of the oxidative enzyme systems for drug metabolism is known to be very low in hepatic microsomes derived from fetal and newborn animals The recent identification of the component members of the microsomal electron transport chain prompted the present study The rate limiting factor in the oxidative drug metabolic process appears to be not the amount of cytochrome P450 (the last component of the chain) but rather the rate of the NADPH mediated reduction of this cytochrome Measurement of these molecular components may explain why activity is low in the neonate and may also elucidate the nature of the regulator(s) of this enzyme system

Activity of the mixed function oxidase system and its components has been measured in fetal neonatal and maternal liver and placenta both in control rabbits and following phenobarbital administration The demethylation of aminopyrine and hydroxylation of triamcinolone and benzpyrene was minimal in fetal and neonatal liver and placenta Activity could be induced by administration of phenobarbital to the pregnant doe A much greater (10 fold) increase in cytochrome P450 content and activity of the NADPH mediated cytochrome reductase resulted from phenobarbital treatment A possible explanation for these findings may be the oxygen dependence of the cytochrome system

Pentti Jouppila & Sakari Suonio (Oulu Finland) *The influence of phenobarbitone given to toxemic and normal gravidas on the serum bilirubin concentration in newborn infants*

The decrease in the bilirubin levels caused by phenobarbitone in newborn infants is believed to be due to increased activity of glucuronyl transferase following the induction of enzymes in liver microsomes In some studies this kind of effect of phenobarbitone has been criticized Furthermore the bilirubin levels in babies of toxemic patients have been found to be lower than in normal cases

In this study 30 toxemic patients received a dosage of 100 mg phenobarbitone per os daily The patients were treated in the Department of Obstetrics and Gynecology of the University of Oulu from 5 to 20 days before delivery Some patients received in addition, other medicaments, e g pentymal dihydralazine, klortalidone or sulphonamides The control material consisted of hospitalized toxemic patients who did not receive any barbiturates The serum bilirubin concentration of newborns was followed daily from 4 to 6 days after delivery

The other group included 20 normal maternal care gravidas who received the same dosage of 100 mg phenobarbitone per os daily from 8 to 18 days before delivery The control material of this group comprised 30 normal gravidas who had received only iron and vitamins during the pregnancy The bilirubin levels in the babies were followed as in the group of toxemic patients

The toxemic and normal groups without any barbiturate therapy have been compared on the basis of daily bilirubin levels in the newborn infants in the discussion In addition the toxemic and normal groups treated with barbiturates have been compared with the control groups

Anno Ojala & Maila Koivisto (Oulu Finland) *Oestrogen given to the mother and its effect on the bilirubin level of the newborn*

In the present study 25 mg oestradiol benzoate was given intramuscularly to 60 mothers on the day b fore the delivery to induce labour owing to post maturity toxaemia or for some other reason Two mothers had twin pregnancies which raised the total of the children of the series proper to 62 The bilirubin content of the blood of these babies was observed for the first 7 days of life There was a control group of 19 mothers whose labour was induced for identical reasons but without oestrogen sensitization This group included one mother with twin pregnancy The total of control children was therefore 20

In the first 3 days the bilirubin levels of the children s blood did not differ significantly but after the third day the levels were considerably higher in the children whose mothers had been sensitized with oestrogen than in the control group children At the age of 5 $^1/_2$ days for example the mean bilirubin content in the blood of the series proper was 11 82 mg/100 ml (S D ±4 49) and of the control group 6 07 mg/100 ml (S D ±2 71) The role of oestrogen in the conjugation of bilirubin is discussed

Tapani Luukkainen (Helsinki Finland) *Effect of ethanol on uterine contractions*

After the observation of Fuchs & Wagner 1963 that alcohol inhibits the release of oxytocin in puerperium the results of two clinical trials on the effect of ethanol in the treatment of threatened premature labor have been reported In New York Fuchs used the intravenous infusions and we use the oral route of the administration of alcohol In our treatment the loading dose consists of 60 ml of 34% alcohol followed by 30 ml dose 15 min after the initial dose which the patients are also advised to take at home on the premature beginning of regular and/or painful uterine contractions After this loading dose the patients are treated by giving at least 3 days 30 ml of alcohol three times per day after the last signs of the threatened premature labor The labor is significantly postponed also after premature spontaneous rupture of membranes It seems that this treatment allows the defence mechanisms of pregnancy to recover if the failure in the basic protection is reversible

The contraindications for the treatment are disturbances in the maternal liver function and the progression of labor over 3 cm of cervical dilatation

Pekka H Pikkarainen & Niels C R Raiha (Helsinki Finland) *The development of alcohol dehydrogenase and its isoenzymes in the human liver*

Ref *Nature* 222 563 1969

Kai O Lindros & Niels C R Raiha (Helsinki Finland) *Development of some enzymes involved in gluconeogenesis in human liver*

Ref *Annales Med Exp Fenn* 47 146 1969

S Obenshain K King P Adam K Raivio K Teramo N Raiha & R Schwartz (Cleveland USA and Helsinki Finland) *Human fetal insulin response to sustained maternal hyperglycemia*

Ref *Pediatric Research* 3 380 1969

Gerhard Gennser & Erik Nilsson (Lund, Sweden) *Regulation of plasma glucose level in human midterm fetus*

Earlier investigations have demonstrated a considerably lower fetal than maternal plasma glucose level in several mammalian species Several recent works seem to indicate a different fetomaternal relationship at midgestation in homo This communication describes determinations of plasma glucose concentration in 30 midterm human fetuses Successive blood samples were obtained from the umbilical vessels after clamping of the cord The average plasma glucose concentration in one umbilical artery within 60 sec after incision of the uterus was 49 and the simultaneous level in a peripheral maternal vein was 86 mg per 100 ml At 3 min after obstruction of the umbilical flow the fetal plasma glucose level had increased markedly the change being up to 28 mg per 100 ml The instantaneous veno-arterial difference in the umbilical plasma glucose concentration approximated 10 mg per 100 ml at the beginning of the asphyctic period The effect of asphyxia on the fetal glycogenolytic liver enzymes was studied The role of the placenta and the ability of the midterm fetus to participate in the regulation of its plasma glucose concentration is discussed

H Ekelund Ulla Hedner & B Astedt (Malmo Sweden) *Fibrinolytic activity in the human fetus*

The material consisted of blood obtained via a catheter in the umbilical artery or by heart puncture, from 74 foetuses 10–32 cm long and extracted on legal grounds from healthy women in the 12th to 24th week of pregnancy

Laboratory studies Determinations were made of fibrinolytic activity on fibrin plates (18 fetuses) euglobulin clot lysis time (8) fibrinolytic split products in serum (49) and in serum with addition of epsilon amino caproic acid (EACA) (54) fibrinogen (17) plasminogen (63) antiplasmin (31) α macroglobulin (42) inhibitors of urokinase activation by plasminogen (19)

Results The fibrinolytic activity was high in all the cases studied The plasminogen content was about 25% α macroglobulin about 40% and the fibrinogen 25–30 of adult values Inhibitors of plasminogen activation were increased and antiplasmin was of adult level Statistical analysis showed that only α macroglobulin increased with the size of the fetus

The results show that the fibrinolytic activity in foetal blood is considerable and that the various factors of the fibrinolytic system develop early The factors presumably form in the foetus and are necessary for maintenance of a patent fetoplacental circulation The findings also argue against the assumption that the hyaline membrane syndrome in prematures is due to a primary deficiency of the fibrinolytic system Defects in the fibrinolytic system in infants with the hyaline membrane syndrome are presumably secondary to other metabolic disorders

The investigation supports rather the clinical and experimental observations showing that the fibrinolytic activity is increased in hypoxic newborns Part of the cause of intracranial haemorrhage in these infants might therefore be explained by hyperfibrinolysis and not by a defective coagulation system Profylactic treatment with EACA of the infants at risk or via treatment of the mother therefore appears to be well founded

Bengt Bengtsson Erik Nilsson & Gerhard Gennser (Malmo Sweden) *Sodium and potassium in human fetal and maternal erythrocytes*

Determinations were made of the sodium potassium and water content of plasma and washed erythrocytes in blood from human midterm fetuses newborns their mothers and from 1-day old infants

No significant differences in the plasma sodium concentration was found between any

wo of the five groups of samples Plasma potassium in successive samples from the umbilical artery in midterm fetuses rose with time after interference with the umbilical circulation The difference between mid term fetal and maternal plasma potassium extrapolated backwards to normal intrauterine conditions i e to the moment before opening of the uterus was less than ±0 5 mE/l

Mean intracellular potassium concentration in the five groups of erythrocytes studied varied between 93 5 and 99 5 mE/kg No significant difference was found between any of the five groups Sodium concentration was higher in erythrocytes from newborn infants and 1 day-old infants than in the other three groups

It is concluded that the fetal plasma potassium concentration at mid term closely reflects the maternal level and that red blood cells from human fetuses at mid term and at term are capable of generating ionic gradients across the cell membrane of the same magnitudes as the adult red blood cells

Bjorn Westin (Danderyd Sverige) *Human fetal response to intrauterine pure tone stimulation*

Studies of the fetal response to acoustic stimuli are of interest for several reasons and are at present concerned with the following questions 1) At what gestational age does the first response occur? 2) Are such fetal responses a measure of the function of the cochlea and its sensory end organ? 3) How do intrauterine responses compare with postnatal auditory tests? 4) Can hearing impairment be detected already in the fetus? 5) Can acoustic stimulation of the fetus evoke a cerebral response? 6) Do fetal responses to acoustic stimuli occur in other species? 7) Are fetal responses to acoustic stimuli of adaptive value? — If so it would be interesting to test this in the human fetus

An external bell shaped plexiglass vibrator in direct contact with the abdominal wall via a liquid interface presented during 1 sec a tone at the ear of the fetus with a frequency of 3 000 Hz (to exclude vibration sense) and a SPL of 110 dB according to intrauterine calibrations Fetal heart rate was recorded with phonocardiogram The presentation of the pure tone was regularly followed by tachycardia and thereafter often by bradycardia before normalization took place (Johansson Wedenberg & Westin 1963) Significant tachycardia following sound stimulation was seen in occasional fetuses in the 22nd week (corresponds well with the development of the cochlea and the sensory end organ) in about 50% of fetuses in the 26th week and in all cases in 28–36 week from estimated conception (Westin 1968) Postnatal auditory tests were consistent with normal hearing in all cases Auro-palpebral reflex 110 dB SPL Awakening from sleep 70 dB SPL 500–4 000 Hz (22 cases)

A high risk material (suspected hereditary deafness) was examined in a similar fashion Thirty fetuses were examined The intrauterine response was normal in all cases but one Similar results were obtained when postnatal auditory tests were performed The fetus that showed no intrauterine response also disclosed hearing impairment postnatally (Johansson Wedenberg & Westin 1970)

Responses to acoustic stimuli can be of adaptive value Gottlieb (1963) has indicated that unhatched young of the wood duck respond to the quacks of the mother with increases in heart rate Shortly after they have all hatched the mother wood duck will leave the nest and make quacking calls to the ducklings in the nest whereupon all the young jump out of the nest and into the water several feet below them This is a behaviour that has no precedent — About 24 hours before hatching the heart beat of the ducklings begins to increase in response to the quack of the mother The increase in heart rate becomes stronger and stronger until just prior to hatching Apparently the ducklings become imprinted to the quack of the parent while they are still in the egg (cf Kramer 1968)

Recently Sakabe Arayama & Suzuki 1969

have recorded the cerebral evoked to acoustic stimuli from the abdominal wall at the 32nd to 38th week of pregnancy A bone vibrator and averaging electronic computer technic was used The typical wave form of the response consisted of four prominent deflections These deflections correspond to the well recognized slow vertex potential to auditory stimulus in young children

Whether the human fetal response to acoustic stimuli is of adaptive value or can be used in improving learning ability remains to be tested

Ritva Hilty Hans Johnell & Bo A Nilsson (Uppsala Sverige) *Oxygen carbondioxide tension and pH in amniotic fluid during maternal hyperoxia and hypoxia in the second trimester*

Goran Zador (Falun Sweden) *Continuous recording of the fetal heart rate and labour activity with the cardiotocograph 8020 A Experiences of one year s use*

Since December 1 1968 the Cardiotocograph 8020 A manufactured by the Hewlett Packard Company has been used at the Department of Obstetrics and Gynecology in Falun

During a period of 1 year from December 1 1968 we have carried out 102 monitoring, selected from 1995 deliveries The frequency of monitoring was consequently 5 1 % The system was completed in April 1969 with scalp electrode for fetal ECG used after the rupture of the membranes

The recorded cases were chosen either because of their belonging to the group high risk pregnancy —first of all overcarriages and toxemia—or because of irregularity of the fetal heart rate during delivery

Our aim has been to judge the practical value of this Cardiotocograph on the level of a Swedish Central Hospital where relatively few specialists have to face a great number of pathological labour conditions and, therefore, all technical means are of great importance

The results have been analysed according to the investigations of Hon Caldeyro Barcia & Hammacher and have been compared with the Apgar score of the newborns one minute after the delivery

Among the 102 recordings 25 pathological patterns have been found Two cases of silent pattern have been observed In both cases the mothers received Ketobemidon chloride (Ketogin) before the monitoring was started Eight registrations of Dips type I were obtained Seven of the mothers were treated conservatively but all the newborns received at least Apgar score 8 which points out that this pattern appears to be harmless In one case with mainly Dips I but also with a few Dips II we carried out Caesarean section An extremely short umbilical cord was found during the operation

Dips II has been found in 15 cases five times together with tachycardia Only three of the newborns had an Apgar score of 8 or more To this group belonged the only stillborn child in the whole material in spite of the fact that Caesarean section was performed In this group another 5 Caesarean sections and 5 vacuum extractions were made Among the newborns we found 6 cases with Apgar score 5–7 and 5 cases with Apgar score less than 5 Three cases of placental insufficiency were verified by pathological examination

According to our preliminary results the method is of great value by giving us increasing possibilities to distinguish alternation of fetal heart rate patterns with good or poor prognosis for the newborn The conventional auscultation does not allow us to make qualitative estimations of any kind

Antti Koivikko (Turku Finland) *Experimental hypothermia in neonatal lambs*

Nine neonatal lambs were anaesthetized with chloralose to prevent shivering during artificial

ypothermia Four lambs which were given the naesthetic for sedation only shivered when ær blood cooled The oxygen consumption ardiac output and heart rate of the non hivering lambs decreased exponentially as the emperature decreased Oxygen consumption f the shivering animals increased from the ontal level and was maintained at a clearly igher level than in non shivering lambs during oolin The cardiac outputs decreased to the ame extent in the shivering lambs as in the non shivering lambs

The latter finding is at variance with that obtained in experiments with adult dogs (Prec 1949) in which shivering during hypothermia caused an oxygen consumption and also an in creased cardiac output The unresponsiveness of the cardiac output of neonatal lambs to in cr ased oxygen consumption resembles the poor response of neonatal lambs to arterial hypoxia as compared to the response of adult sheep (Korvikko 1969) The basic reason for this phenomenon remains to be determined

Gunnlaugur Snaedal & Gunnar Biering (Rey kjavik Iceland) *Prevention of Rh isoimmuni zation in Iceland*

When it became apparent in 1968 that isoim munization to the Rhesus factor could be pre vented by injection of anti D antibody prepara tions for prophylactic measures on a nation wide basis in Iceland were instituted

A decision was made to treat all nonsensit ized Rh negative women multiparae as well as primiparae Furthermore it was decided to in clude in the programme all Rh negative women with 1 gal and spontaneous abortions as far as they could be reached

With a total population of 203 000 it was felt that such prophylactic measures could be accomplished with relative ease

Approximately 700 Rh negative women are expected to deliver per year in the near future This again means approximately 450 injec tions of anti D immune globulin per year to effect a good Rh prophylaxis in the country

The bloodbank in Reykjavik in close co-operation with the University Hospital matern ity clinic will be the center from which the Rh prophylaxis will be directed

All blood typing of pregnant women in the country will be performed at the bloodbank in Reykjavik which thus gains informations con cerning all Rh negative pregnant women in the country at each time Those who prove to be Rh negative will be followed with antibody screening tests in the later part of pregnancy

When Rh negative nonsensitized women deliver Coombs test and bloodtyping for Rh are performed on placental blood and those women who can benefit from an anti D im mune globulin injection will receive it

In addition to the maternity clinics in Rey kjavik where approximately 50 of all deliv eries in the country occur six district hospitals strategically located in different areas of the country will be equipped to do Coombs testing and Rh typing of placental blood

In cases of spontaneous or legal abortions bloodtyping will be encouraged so that those who prove to be Rh negative can receive anti D immune globulin

Preparations for the abovementioned pro-gramme were completed in November 1969 and the programme was officially launched on December 17 1969

Martti Kauppinen Telma Koli & Carl Erik Raiha (Helsinki Finland) *Perinatal mortality in Helsinki*

Since vaccinations and use of antibiotics have eliminated most of the mortality during child hood the perinatal mortality and the invalidat ing events during this period have become the central problem in pediatrics The present peri natal mortality in Finland is about 2 5% equally late fetal death and deaths during the first neonatal week The mortality *during the first week* equals the mortality during the fol lowing 783 weeks of life which makes 15 years During fetal development and peri

natal period induced invalidity can at present time not be determined in Finland However Herlitz & Redin in Östergotland, Sweden have shown that 1 24% of the total child population between 0–15 years are invalids More than 3/4 of these are caused by evils during prenatal and perinatal life that is mental retardation cerebral palsy epilepsy and malformations (anatomical ch mical and immunological) 3/4 of the perinatal mortality is due to low birth weight (below 2 5 kg) Mental retardation is in 90% caused prenatally and during perinatal period (Øster) Cerebral palsy is 60 times more frequent in prematures than in babies with a birthweight over 3 kg (MacDonald) and the *incidence of malformations is 10 to 20 times* more frequent in prematures than in fullborn (Butler & Bonham) dependent on the birth weight

At the prenatal clinics in Helsinki with about 7 000 to 9 000 pregnancies a year the perinatal mortality has decreased from 2 6–2 7% during the period 1955–59 to 1 8 1960–1963 1 6 1964–66 and to 1 2% 1967 in the group participating in the heart volume measurements Pregnant women are advised to rest horizontally some hours daily if the heart volume is small This group consists of about 50 000 pregnancies In the group in which participation in this service is refused the perinatal mortality during the period 1960–1967 has been 3 5% the number of pregnancies in the group is about 10 000

The decrease in the perinatal mortality depends mainly on *declining number of small* premature babies This development and the distribution of the cases in different groups known as associated with prematurity and low birth weight will be discussed

Mike Kellum John Gentz & Bengt Persson (Stockholm Sweden) *Catecholamine induced lipolysis and calerogenesis in newborn piglets*

The relationship between catecholamine induced lipolysis and calerogenesis was studied in neonatal piglets (5 hours to 13 days old) in order to define the contribution of norepinephrine (NE) to the thermogenic response of the neonate Unlike human infants, piglets lack brown fat and therefore allow differentiation of the roles of brown and white fat in this response

NE infusion into piglets for 25 min (2 0 μg/kg min) resulted in a small (mean 5 9%), variable (from −2 2 to 15 2%) increase in oxygen consumption (VO) that was not correlated with the increases in free fatty acids and glycerol nor with those of glucose or lactate The increases of VO seen after similar doses of epinephrine were of similar magnitude *Administration of nicotinic acid prior to the second* NE infusion resulted in a suppression of lipid mobilization but no decrease of the comparative stimulated increase in VO Hyperglycemia and hyperlacticacidemia persisted In an older (17 days) animal however inhibition of lipolysis did result in a fall in stimulated VO

The results show that lipid mobilization may occur without increases of VO and vice versa Lipolysis per se therefore does not appear to be a primary determinant of NE induced calerogenesis It is suggested rather that utilization *of FFA mobilized from white adipose tissue* may be a more critical factor in determining the lipolytic related component of calerogenic effect of NE and that this may change with postnatal age

John Gentz & Bengt Persson (Stockholm Sweden) *The importance of feeding for the rise in oxygen consumption during the first days of life in newborn pigs*

Factors influencing oxygen consumption (VO) and rectal temperature (T_r) were studied in piglets aged 0 to 18 days Fed animals 0 to 24 *hours old had significantly higher VO and* T_r than comparable groups of unfed piglets Fed animals 1 to 18 days old showed no significant

rise in VO and T_R although there were significant increases in body weight and decreases in thermoneutral environmental temperature. Correlations between VO and T_R measured in the zone of thermoneutrality were present in animals 1 to 18 days old but absent in newborn unfed piglets.

When sow's milk was given to previously unfed piglets there was a marked rise in VO and T_R both correlated with the amount of milk given. The rise in VO always preceded the increase in T_R indicating that T_R per se did not determine VO.

It is concluded that during the first 24 hours of life food intake has a relatively greater effect on VO and T_R than factors such as age, weight and moderate changes in the environmental temperature. Therefore comparisons of VO may be misleading if the nutritional status of the animals vary. The present definition of thermoneutral zone in this period is also questioned.

B. Thalme & H. Shelley (Oxford, England). *Body temperature and plasma free fatty acid in normal and anoxic newborn rabbits in the first hour after delivery.*

Newborn rabbits delivered by Caesarean section were placed in a perspex chamber immersed in a thermostatically-controlled waterbath. Prewarmed AIR or N was flowing through the chamber. At regular intervals one newborn rabbit was removed from the chamber, the deep colonic temperature measured and a blood sample obtained by heart puncture.

The plasma FFA level increased in AIR at 31 C within 10 min but remained unchanged in a warmer environment. The deep colonic temperature fell below 35 C within 5–10 min of delivery. Newborn rabbits delivered into N at 31 C showed a similar rate of cooling, but no rise in plasma FFA. Glucose given intraperitoneally resulted in a normal FFA rise and injection of lactic acid gave a similar but nonsignificant lower rise. The plasma insulin levels did not differ in AIR or N and no correlation was obtained between insulin and FFA.

The postnatal rise in FFA in cool AIR and the absence of this response in N could be due to the depressant effect of anoxia on the central nervous system.

Niels Raiha

BOOK REVIEWS

H Olbing *Harnwegsinfektion und Harnbefund bei Kindern* Georg Thieme Verlag Stuttgart 1969 112 pp DM 34 —

The title of this book is somewhat misleading as its contents is limited to a survey of different methods of diagnosing urinary tract infections by examining urine Bacteriological diagnostics are penetrated and the value of two screening-methods (TTC test and nitrite test) is estimated The author discusses what there is to be gained in looking for leucocytes erythrocytes cylinders and proteins in urine Like Braude *et al* (Brit Med J 1967) he is of the opinion that the number of false diagnoses can be diminished by making different methods of urine examinations complementary to each other

The book is small covering 89 pages Its ambitious and extensive literature list containing 631 references is perhaps the greatest merit of the book

Lennart Righard

Werner Isler *Akute Hemiplegien und Hemisyndrome im Kindesalter* George Thieme Verlag Stuttgart 1969 207 pp DM 69 —

This book gives a comprehensive study of the acute hemisyndromes in childhood The author describes 114 patients 80 with thorough case reports the other in tabulated form About half the cases are illustrated with excellent X ray pictures and explaining sketches of the lesions Unfortunately the very detailed descriptions make the book inadequate in surveyability Each subgroup is described but there is no survey of differential diagnosis which would have been very helpful in this difficult syndrome where often prompt diagnosis is important It is quite clear from reading this book that much can be done by modern radiology to throw light upon the acute infantil hemiplegia of obscure origin

Ingrid Bjerre

Th Baumann & R Frey (eds) *Epilepsie im Kindesalter* Pädiatrische Fortbildungskurse für die Praxis Vol 26 S Karger Basel and New York 1969 136 pp DM 33 —

This book about Epilepsy in Childhood is edited by E Rossi the well known pediatric professor of Bern Many authorities are behind the ten chapters—all in German and one in French Of great clinical value are the perspicuous papers about anti-epileptic therapy and about the side effects of anti-epileptic drugs For the more theoretically interested there are papers dealing with genetical and patophysiological problems of child epilepsia The contents of the book seems to be quite up to date

It is a handy book with a very readable collection of papers

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1st Argentine Congress of Neonatology 1st Argentine Meeting of Pediatric Nursery will be held at Mar del Plata Argentine December 3 to 6 1970 Main subject The high risk new born infant clinical and surgical aspects

For further information write to

Fundación Hospitalaria
Secretary General
Dr José Ricardo Pineyro
Uruguay 937—Capital
Buenos Aires Argentine

Acta Pædiat Scand 59 605–612 1970

RENAL FUNCTION DURING HYDROPENIA AND WATER DIURESIS IN CHILDREN WITH RECURRENT URINARY TRACT INFECTIONS

A APERIA U BERG and O BROBERGER

From the Department of Paediatrics S:t Görans Hospital Stockholm Sweden

Recurrent urinary tract infection is the predominant disease of the kidney The majority of patients with this condition will finally recover but a few will despite intensive treatment finally develop renal insufficiency The predisposing factors for this development are still largely unknown In order to predict the course of recurrent urinary tract infection a definition of the specific pathophysiology is urgently needed Previous studies on this subject (16 17 12) are sparse and have generally been carried out in unspecified adult materials which means that the disease might have been complicated by for instance arteriosclerotic changes Functional studies of nonobstructive recurrent urinary tract infection in childhood have so far only demonstrated that reduction of urinary concentrating capacity often occurs in acute disease (27) It has also been shown that these functional changes will rapidly be restituted following recovery of the infection (28) Those studies have however been limited to determination of the urine osmolality after 16–17 hours of food and fluid deprivation and administration of vasopressin The present report dealing with a well defined material of children with recurrent nonobstructive urinary tract infections is therefore an attempt to define further the pathophysiology of this disease The study has primarily been aimed to evaluate glomerular filtration rate urinary sodium excretion and concentrating and diluting capacity For this purpose the patients were studied during the transition from hydropenia to water diuresis

Supported by the Swedish Research Council Grant B67 19X 2049-01A

Abbreviations V diuresis GFR glomerular filtration rate C_{Na}/C_{In} % of filtered sodium excreted $T^c_{H_2O}$ free water reabsorption (osmolar clearance—urine flow) C_{H_2O} free water clearance (urine flow—osmolar clearance)

MATERIAL

Seventeen patients aged 3 ½–14 ½ years were studied None of the patients had been submitted to selective renal functional studies 1–2 years earlier Those studies have been reported elsewhere (4) All the patients had previous histories of urinary tract infection The diagnosis had been confirmed at least on one occasion with urine culture Most of the patients studied had 2–3 culture confirmed infections yearly In no case were there any clinical or bacteriological signs of infection at least 2 months prior to the study All the patients had normal blood pressure None of them had azotemia acidosis hypocalcemia or hyperpotassemia In none of the patients were there any signs or symptoms of nephropathies other than urinary tract infections

An intravenous pyelography (IVP) was carried out in all the patients within a period of at most 1 year to the reported study A complete description of the various types of radiological changes found in this material has already been given elsewhere (4) The patients in group I had normal IVPs (both parenchyma and pelvis evaluated) The patients in group II had renal parenchymal changes defined in Table 1 The clinical histories of the patients in group I and group II did not differ significantly

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Table 2 *The glomerular filtration rate and urinary sodium excretion during hydropenia in patients with normal IVPs*

Patients	GFR (ml/min/ 1 73 m² b s)	$U_{Na}V$ (μEq/min/ 1 73 m² b s)	C_{osm}/C_{In} (-)
Group I			
L I	114	136	0 89
A I	110	160	1 08
A K	100	119	0 83
J H	100	173	1 20
C H	[illegible]	158	1 25
A K F	[illegible]	114	0 95
Mean	100	143	1 03
S E M	4 6	9 8	0 22
Normal adults	97	140	1 01
S E M	3 3	12 5	0 09
p	0 7–0 6	0 8–0 7	>0 9

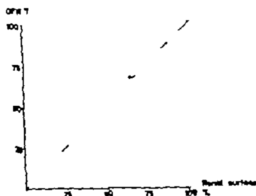


Fig 1 The relationship between the renal surface and the GFR in all the patients with pathological IVPs The renal surface has been calculated as percentage of the average renal surface of two normal subjects of corresponding age and sex The GFR is also given in percentage of the normal GFR for the patient's body surface The dashed line represents the theoretical 1/1 relationship between GFR and renal surface

nificant reduction of the GFR (range 17 to 80 ml/min/1 73 m b s) In Fig 1 the relationship between the GFR and the size of the renal parenchyma has been analyzed in this group of patients The size of the renal parenchyma has been roughly estimated by one dimensional surface calculation In none of the patients however was there any evidence of unusual disproportion between the dimensions The kidney size has been calculated as percentage of the average kidney size of two normal subjects of corresponding age and sex The figure demonstrates a good relationship (within 15%) for 6 of the 11 patients a complete lack of relationship in 5 of the 11 patients one of whom having a much too high GFR one demonstrating a 25% higher GFR than the estimated renal size and the other 3 a too low GFR The total sodium excretion in group II was within one standard deviation of the values from the healthy volunteers in all but 1 patient (I A) The fractional sodium

Table 3 *The glomerular filtration rate and urinary sodium excretion during hydropenia in patients with pathological IVPs*

Patients	GFR (ml/min/ 1 73 m b s)	$U_{Na}V$ (μEq/min/ 1 73 m² b s)	C_{osm}/C_{In} ( )
Group II			
A B	10	195	1 37
C K	90	116	1 00
A K	86	159	1 29
A E	80	112	0 98
S W	77	194	1 79
J J	72	107	1 03
E M L	67	173	1 79
I A	67	414	4 51
G B	58	83	1 00
I L L	33	147	3 36
K H	17	83	3 36

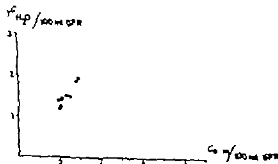


Fig 2 The relationship between free water reabsorption ($T^c_{H_2O}$) and osmolar clearance (C_{osm}) in all the patients studied Both parameters are related to the GFR. Open circles represent the patients with normal IVPs (group I) and filled circles represent the patients with pathological IVPs (group II)

Table 1 *The radiological appearance of the kidneys in patients in group II*

Patient	Left kidney	Right kidney
C K	Moderately reduced in size Even in shape Calices normal	Moderately reduced in size Even in shape Calices normal
A K	Small in size Uneven in shape Calices deformed	Normal in size Even in shape Calices normal
G B	Moderately hypertrophied Even in shape Calices normal	Small in size Even in shape Calices deformed
J J	Moderately reduced in size Even in shape Small calcifica tions Calices normal	Moderately reduced in size Even in shape Small calcifica tions Calices deformed
S W	Small in size Uneven in shape Calices deformed	Normal in size Even in shape Calices normal
I L L	Small in size Even in shape Calices deformed	Normal in size Uneven in shape Calices deformed
A B	Small in size Uneven in shape Small calcifications Calices deformed	Moderately reduced in size Uneven in shape Calices normal
I A	Normal in size Uneven in shape Calices normal	Small in size Uneven in shape Calices deformed
A E	Moderately reduced in size Uneven in shape Calices deformed	Small in size Uneven in shape Calices deformed
E M L	Small in size Even in shape Calices deformed	Normal in size Even in shape Calices normal
K H	Small in size Uneven in shape Calices deformed	Small in size Uneven in shape Calices deformed

METHODS

All the patients were studied during the transition from hydropenia to water diuresis At the beginning of the study the patients were deprived of water for 17 hours Following 2–3 clearance periods during hydropenia water diuresis was induced by allowing the patients to drink water in an amount correspond ing to 2–2 5 of the body weight and thereafter in amounts slightly exceeding the diuresis every 30 min When water diuresis was believed to be complete i e when diuresis was again constant usually within 75 to 90 min another 2–4 clearance periods were carried out All the patients tolerated the study well and were cooperative

Standard clearance techniques were used (24) This includes a continuous infusion of 10 inulin (Lae vasar Gesellschaft) 0 001 g/min/kg b w after a prime dose of 0 05 g/kg b w The equilibration time was 45–60 min For urine sampling a double lumen poly ethylene catheter was used enabling continuous suc tion Blood samples were taken in the middle of each urine sampling period from an indwelling needle in a peripheral vein During the hydropenic stage urine was collected in 30–45 min periods During water diuresis the periods were of about 15 min duration

Urine and blood samples were analyzed with re gard to the concentration of inulin sodium and osmolality The chemical analyses of inulin were carried out according to the method of Heyrowski (10) sodium in an Eppendorff flame photometer and osmolality cryoscopically with a Knauer microosmo meter

RESULTS

Glomerular filtration rate and urinary sodium excretion

Tables 2 and 3 demonstrate the glomerular filtration rate (GFR) per 1 73 m body sur face and the urinary sodium excretion ex pressed in absolute values as well as in per centage of filtered load All values are re corded during hydropenia Table 2 contains the patients with normal IVPs (group I) and Table 3 the patients with renal parenchymal changes (group II) The patients with normal IVPs have been statistically compared to a group of healthy young volunteers The re sults from the latter group have been reported in detail elsewhere (3) The patients with re current urinary tract infections but with nor mal IVPs did not significantly differ from the healthy volunteers with regard to the GFR, the total and the fractional sodium excretion

The patients with pathological appearance of the IVPs (Table 3) were considered too heterogenous with regard to the degree of renal parenchymal changes to be statistically worked up Eight of the 11 patients demonstrate sig

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C H	88	158	1 23
A K F	86	114	0 95
Mean	100	143	1 03
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SEM	3 3	12 5	0 09
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Table 3 *The glomerular filtration rate and urinary sodium excretion during hydropenia in patients with pathological IVPs*

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A B	102	195	1 37
C K	98	116	1 00
A K	86	139	1 79
A E	80	112	0 98
S W	77	194	1 79
J J	77	107	1 00
E M L	67	173	1 79
I A	67	414	4 53
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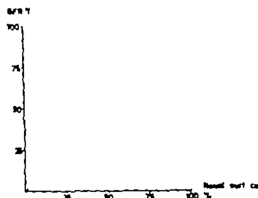


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of the average kidney size of two normal subjects of corresponding age and sex The figure demonstrates a good relationship (within 15) for 6 of the 11 patients a complete lack of relationship in 5 of the 11 patients one of whom having a much too high" GFR one demonstrating a 25 higher GFR than the estimated renal size and the other 3 a too low GFR The total sodium excretion in group II was within one standard deviation of the values from the healthy volunteers in all but 1 patient (I A) The fractional sodium

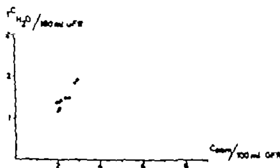


Fig The relationship between free water reabsorption ($T^c_{H_2O}$) and osmolar clearance (C_{osm}) in all the patients studied Both parameters are related to the GFR Open circles represent the patients with normal IVPs (group I) and filled circles represent the patients with pathological IVPs (group II)

Table 1 *The radiological appearance of the kidneys in patients in group II*

Patient	Left kidney	Right kidney
C K	*Moderately reduced in size* Even in shape Calices normal	*Moderately reduced in size* Even in shape Calices normal
A K	Small in size Uneven in shape Calices deformed	Normal in size Even in shape Calices normal
G B	Moderately hypertrophied Even in shape Calices normal	Small in size Even in shape Calices deformed
J J	Moderately reduced in size Even in shape Small calcifications Calices normal	Moderately reduced in size Even in shape Small calcifications Calices deformed
S W	Small in size Uneven in shape Calices deformed	Normal in size Even in shape Calices normal
I L L	Small in size Even in shape Calices deformed	Normal in size Uneven in shape Calices deformed
A B	Small in size Uneven in shape Small calcifications Calices deformed	Moderately reduced in size Uneven in shape Calices normal
I A	Normal in size Uneven in shape Calices normal	Small in size Uneven in shape Calices deformed
A E	Moderately reduced in size Uneven in shape Calices deformed	Small in size Uneven in shape Calices deformed
E M L	Small in size Even in shape Calices deformed	Normal in size Even in shape Calices normal
K H	Small in size Uneven in shape Calices deformed	Small in size Uneven in shape Calices deformed

METHODS

All the patients were studied during the transition from hydropenia to water diuresis At the beginning of the study the patients were deprived of water for 17 hours Following 2–3 clearance periods during hydropenia water diuresis was induced by allowing the patients to drink water in an amount corresponding to 2–2 5 of the body weight and thereafter in amounts slightly exceeding the diuresis every 30 min When water diuresis was believed to be complete i e when diuresis was again constant usually within 75 to 90 min another 2–4 clearance periods were carried out All the patients tolerated the study well and were cooperative

Standard clearance techniques were used (24) This includes a continuous infusion of 10% inulin (Laevosan Gesellschaft) 0 001 g/min/kg b w after a prime dose of 0 05 g/kg b w The equilibration time was 45–60 min For urine sampling a double lumen polyethylene catheter was used enabling continuous suction Blood samples were taken in the middle of each urine sampling period from an indwelling needle in a peripheral vein During the hydropenic stage urine was collected in 30–45 min periods During water diuresis the periods were of about 15 min duration

Urine and blood samples were analyzed with regard to the concentration of inulin sodium and osmolality The chemical analyses of inulin were carried out according to the method of Heyrowski (10) sodium in an Eppendorff flame photometer and osmolality cryoscopically with a Knauer microosmometer

RESULTS

Glomerular filtration rate and urinary so
excretion

Tables 2 and 3 demonstrate the glome filtration rate (GFR) per 1 73 m body face and the urinary sodium excretion pressed in absolute values as well as in centage of filtered load All values ar corded during hydropenia Table 2 co the patients with normal IVPs (group I) Table 3 the patients with renal parench changes (group II) The patients with no IVPs have been statistically compared group of healthy young volunteers Th sults from the latter group have been rep in detail elsewhere (3) The patients wi current urinary tract infections but with mal IVPs did not significantly differ fro healthy volunteers with regard to the (the total and the fractional sodium exc

The patients with pathological appea of the IVPs (Table 3) were considered heterogenous with regard to the degree of parenchymal changes to be statistically w up Eight of the 11 patients demonstrate

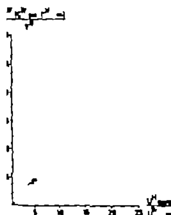


Fig 6 The relationship between expected (abscissa) and actual (ordinate) dilution of urine during the transition from hydropenia to water diuresis in the patients with pathological IVPs (group II) when differences in osmotic clearances have been corrected for Symbols see Fig 5 Those results that are beyond the normal range i e implying a defect in the dilution have been indicated by filled circles

Table 4 *The mean values with one standard deviation for the glomerular filtration rate and fractional sodium excretion during hydropenia and water diuresis in the different groups The difference of mean is also listed*

	Hydropenia	Water diuresis	Mean difference
	GFR (ml/min/1 73 m² b s)		
Group I	100 ± 11	112 ± 13	12 ± 6 $p < 0.01$
Group II *a*	64 ± 28	94 ± 29	30 ± 16 $p < 0.01$
Group II *b*	73 ± 26	80 ± 20	7 ± 8 $0.4 < p < 0.5$
	C_{Na}/C_{In} ( )		
Group I	1 03 ± 0 17	1 50 ± 0 53	0 48 ± 0 54 $0.05 < p < 0.1$
Group II *a*	1 53 ± 0 95	2 19 ± 0 98	0 66 ± 0 69 $0.05 < p < 0.1$
Group II *b*	2 47 ± 1 42	2 64 ± 1 44	0 18 ± 0 28 $0.2 < p < 0.3$

Statistically significant difference

…ion had been corrected for Since all the patients reported were studied during the transition from hydropenia to water diuresis the same analysis could be carried out with the present results (Figs 5 and 6) It is apparent from Fig 5 that all the patients with normal IVPs are able to dilute the distal tubular fluid more during water diuresis i e the actual dilution of urine (ordinate) exceeds the expected dilution (abscissa) even when variance in osmolar clearance is corrected for The results from the patients with renal parenchymal changes (group II) are however more variable (Fig 6) Five of those patients (filled circles) were apparently unable to increase their diluting capacity while the rest of the patients (open circles) respond to water diuresis in the same way as the patients with normal IVPs and the healthy adults On the basis of those results the patients in group II have been divided into two subgroups II *a* and II *b*

Changes in glomerular filtration rate and urinary sodium excretion during the transition from hydropenia to water diuresis

Table 4 demonstrates the average changes in GFR and urinary sodium excretion during the transition from hydropenia to water diuresis in group I II *a* and II *b* The individual patterns of the GFRs are depicted in Fig 7 In the study of healthy adults the GFR was found to be slightly but significantly increased in water diuresis The patients with normal IVPs (group I) showed the same *pattern* The patients with parenchymal changes who were able to increase their diluting capacity during water diuresis i e belonging to group II *a* also increased their GFR significantly In fact most of these patients exhibited a steeper increase

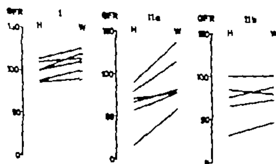


Fig 7 Changes in GFR during the transition from hydropenia to water diuresis for patients with normal IVPs (group I) patients with pathological IVPs with normal dilution (group II *a*) and patients with pathological IVPs with defect dilution (group II *b*)



Fig 3 The relationship between fractional sodium excretion (C_{Na}/C_{In}) and urine osmolality (U_{Osm} mO/l) during maximal hydropenia All the patients studied are included in the figure Open circles represent the patients with normal IVPs (group I) and filled circles represent the patients with pathological IVPs (group II)

excretion was markedly increased in 3 of the patients on the upper limit in two and normal in the rest of the patients

Concentrating capacity

The urinary concentrating capacity is represented both in terms of free water reabsorption and maximal urinary osmolality In Fig 2 the free water reabsorption has been related to the osmolar clearance All the patients (i e from both groups) are included in the figure The relationship is the same as that found in healthy adults (9 19) in all but three patients Those 3 patients who all belong to group II have an elevation of the osmolar clearance but do not exhibit the expected increase in free water reabsorption that is found when osmolar clearance is raised with mannitol or saline diuresis (9, 19)

In order to reveal further the mechanism of reduced concentrating capacity in some of the patients the relationship between urinary osmolality and the fractional sodium excretion has been examined (Fig 3) It is quite clear that a marked increase in fractional sodium excretion is always accompanied by a marked fall in urinary osmolality

Diluting capacity

Fig 4 demonstrates the diluting capacity as expressed by free water clearance in relationship to osmolar clearance in all the patients studied The values are within the range of that found in healthy adults (3)

Previous studies in healthy adults have demonstrated that the fluid of the distal tubule is more diluted during water diuresis than during hydropenia (3) This finding was accomplished by comparing actual and expected dilution of urine after discrepancies in osmotic

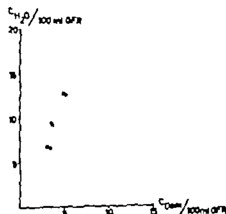


Fig 4 The relationship between free water production (C_{H_2O}) and osmolar clearance (C_{Osm}) during water diuresis Both parameters are related to the GFR All the patients studied are included in the figure Open circles represent the patients with normal IVPs (group I) and filled circles represent the patients with pathological IVPs (group II)

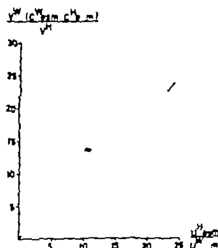


Fig 5 The relationship between expected (abscissa) and actual (ordinate) dilution of urine during the transition from hydropenia to water diuresis in the patients with normal IVPs (group I) when differences in osmotic clearances have been corrected for V^W urine flow ml/min during water diuresis V^H urine flow ml/min during hydropenia U^W urine osmolality during water diuresis U^H urine osmolality during hydropenia C^W = osmolality clearance during water diuresis C^H = osmolality clearance during hydropenia The dashed line represent the theoretical 1/1 relationship between the expected and actual dilution of urine

ceeed that the diluting capacity is primarily a function of the active sodium reabsorption in the distal tubule. It has been implied by several authors that the intrarenal regulation of the blood supply is by some means linked to the sodium reabsorption at the site of the macula densa, i.e. the early distal tubule (1, 2, 7, 25, 26). Thus it has been suggested that impairment of sodium reabsorption at this site will cause a renal vasoconstriction (1, 2). It is therefore of interest that the patients that were unable further to dilute the urine, i.e. increase the distal sodium reabsorption during water diuresis, constitute the only group where the GFR was not significantly increased during water diuresis. Thus the results from this study are suggestive of a change in the renal vascular tonus secondary to subtle changes in the tubular handling of sodium in some of the patients with recurrent urinary tract infections.

Previous studies on the functional damage following episodes of urinary tract infection have mostly been made in adult patients (12, 16, 17). Detailed descriptions of the radiological appearances of the patients are not given. Reduction of the GFR has been the predominant lesion. The glomerular tubular balance has been found to be preserved as far as the secretion of PAH (16, 17) and reabsorption of glucose (20) are concerned unless the glomerular filtration rate is reduced beyond 15 ml/min/1.73 m. b.s. The results have been interpreted to mean that in recurrent urinary tract infections there is a progressive complete destruction of the nephrons. The functional findings in children with recurrent urinary tract infections only partially agree with the findings in adults. In children functional changes can only be found if the IVP appearance is abnormal. The renal function can however not with any certainty be predicted by the radiological size of the kidney. Reduction of the GFR appears to be the dominant lesion also among children. Naturally this is partially due to an anatomical destruction of the nephrons. As has been suggested above, a vasoconstriction related to changes in distal tubular sodium reabsorption might contribute to the low GFR during hydropenia. The glomerular tubular balance as far as sodium is concerned appears to be reset at a higher level of glomerular filtration rate than what has been reported on the glomerular tubular balance for glucose. In agreement with Kleeman *et al.* (12) the patients with the most severe reductions of the GFR were unable to concentrate the urine maximally. In contrast however to what has been found in adults, children with recurrent urinary tract infections appear to preserve their diluting capacity during water diuresis to a larger extent.

SUMMARY

The glomerular filtration rate (GFR), urinary concentrating and diluting capacity and sodium excretion have been determined in 17 children with previous histories of urinary tract infection confirmed by urine cultures. There were no signs or symptoms of infection at the time of the study. Renal function was found to be normal in patients with normal intravenous pyelographies (IVPs). In the patients with renal parenchymal changes a wide variety of functional changes could be found. Reduction of the glomerular filtration rate was the predominant lesion. The GFR could however not be predicted by the IVP appearance. Most of the patients were able to increase the GFR during water diuresis. The glomerular tubular balance for sodium was reset in 3 of the patients as manifested by an increased C_{Na}/C_{I}. The concentrating capacity was also reduced in those 3 patients, indicating an impairment of the sodium reabsorption in the loop of Henle. All the patients were able to dilute the urine normally during water diuresis but 5 of the patients were unable to increase their diluting capacity during the transition from hydropenia to water diuresis. It is noteworthy that only those patients were unable to increase the GFR significantly during the transition from hydropenia to water diuresis.

than the patients with normal IVPs In contrast the group of patients with renal parenchymal changes who were unable to increase their diluting capacity during water diuresis, i e belonging to group II b did not have any significant increase of the GFR

The fractional sodium excretion increased slightly in all groups during the transition from hydropenia to water diuresis This observation is in harmony with that made in healthy volunteers (3)

DISCUSSION

The patients with normal IVPs demonstrate a wide variety of clinical histories Two of the patients had the history of at most one infection three of the patients had the history of one to three urinary tract infections yearly and one of the patients had had several episodes yearly of bacteriuria high fever and unilateral abdominal pain radiating dorsally Yet one of the most consistent findings of this study was the completely normal renal function found in those patients The lack of significant difference in the results of children with recurrent urinary tract infections but with normal IVPs and the results of healthy adults is also of interest since it confirms the findings of other investigators that renal function after the age of 2 years appears to be fully developed and could well be related to body surface (8 18 21)

In all the patients with pathological appearance of the IVPs (group II) one or more of the parameters determined were impaired Reduction of the GFR appeared to be the dominant change in renal function Previous unilateral studies (4) have demonstrated that patients with recurrent urinary tract infections associated with renal parenchymal changes often demonstrate subclinical reductions of GFR The unilateral studies also revealed that the filtration rate could not be simply related to the size of the renal parenchyma The same lack of relationship could be demonstrated in 5 of the 11 patients in group II

Three of the patients in group II exhibited a marked increase in the fractional sodium excretion All those patients had low filtration rates (67, 33 and 17 ml/min/1 73 m bs respectively) It is well established that when the functioning population of nephrons is reduced either by ligation of branches from the renal artery (22) by progressive renal disease (15) or by surgical removal (23) the urinary sodium excretion will increase This reset of the level of glomerular tubular balance also demonstrated by the patients in this material is thought to be necessary for the control of fluid and sodium balance during declining renal function

The finding of a reduced concentrating capacity indicates an impairment of the active sodium reabsorption in the loop of Henle the main determinator of the counter current system (5) The concomitant finding of an increased fractional sodium excretion in those patients where the concentrating capacity was reduced supports this assumption Since pyelonephritis is primarily a disease of the renal medulla this localisation of functional damage could almost be predicted A certain vulnerability of the active tubular sodium reabsorption during renal disease is also to be expected since this function is by far the most energy demanding process of the kidney (11, 13) It is thus somewhat remarkable that the impairment of the concentrating capacity the dominant functional change during acute urinary tract infection (6 14 27 28) is so readily reversible that it was only found in three of the patients studied

The urinary diluting capacity as far as free water production is concerned was found to be normal in all the patients studied Thus it can be expected that all the patients could adequately handle a given load of water Yet if the results are analyzed more thoroughly it is found that five of the patients are unable to increase their diluting capacity in the distal tubule during the transition from hydropenia to water diuresis This finding might have important implications for the control of renal hemodynamics in those patients It is generally

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TREATMENT OF CYSTINOSIS WITH A DIET POOR IN CYSTINE AND METHIONINE

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Cystinosis is an autosomal recessive inborn error of metabolism in which cystine because of still unknown mechanisms is accumulated in most organs and tissues giving rise to a de Toni–Debré–Fanconi syndrome photophobia and sometimes liver cirrhosis

The disease has been extensively reviewed in several reports (2 4 5 10 14 16 17 18 22)

The classic treatment of cystinosis is symptomatic being directed against the rickets the hypopotassemia and the acidosis Most often large doses of vitamin D and a solution of potassium and sodium citrate and citric acid are used In spite of improvement during this therapy (3 12) the poor prognosis is not altered

Penicillamine has been used as a substance which might reactivate the inactivated SH dependent enzymes and increase the excretion of cystine (2 6 9 15 16 18) Some authors have been able to lower the high blood level of pyruvate and alphaketoglutarate in their cystinosis patients but on the whole the results of such therapy have been unconvincing Effective treatment with an anabolic steroid has been described in a single case (21)

As methionine is largely converted to cystine in the body it seemed obvious to try to reduce the storage of cystine with a diet poor in methionine (16) It seems that a diet which is poor in both cystine and methionine (1 13 19) might achieve a better result even though this diet has been tried by some without objective evidence of any improvement (18)

Bauer & Antener (1) obtained a negative sulphur balance and improved both the tubular and glomerular function in a 2 ¹/ year old boy with a diet poor in cystine and methionine based on lentils powder Moreover the treatment consisted of anabolic steroid choline chloride as a methyldonor vitamin D and periodic electrolyte supplement

In the following report we will present our experiences in the treatment of 2 children with cystinosis They were followed up for a year during treatment as proposed by Bauer & Antener (1) and the preliminary results have given us grounds for moderate optimism

CASE REPORTS

Case I

Family history L. R M a boy was the youngest child of four in a family with healthy but related parents (Fig 1) The eldest child his sister is described below as case 2 His youngest brother died from severe dehydration when 2 / years old An amino acid analysis on kidney tissue taken during the post mortem investigation showed storage of cystine in great quantities His eldest brother is healthy

Case history The patient was born December 22nd 1965 Pregnancy and delivery were uncomplicated His birth weight was 4000 g and his crown heel length

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REFERENCES

1 Aperia A C *Studies on the regulation of renal hemodynamics* Stockholm 1968
2 — Tubular sodium reabsorption and the regulation of renal hemodynamics The effect of chlorothiazide on renal vascular resistance *Acta Physiol Scand* 75 360 1969
3 Aperia A C Broberger O Ericsson N O & *Feychting H Renal function in man during* transition from hydropenia to water diuresis with superimposed mannitol load *Acta Physiol Scand 77 429 1969*
4 Berg U Aperia A C Broberger O Ekengren K & Ericsson N O Relationship be *tween glomerular filtration rate and radiological* appearance of the renal parenchyma in children *Acta Paediat Scand* 59 1 1970
5 *Berliner R W & Bennett C M* Concentration of urine in the mammalian kidney *Amer J Med 42* 777 1967
6 Brod *J* Chronic pyelonephritis *Lancet 270* 973 1956
7 Cortney M A Nagel W & Thurau K A *micropuncture study of the relationship between* flow rate through the loop of Henle and sodium concentration in the early distal tubule *Pflugers Archiv Ges Physiol 287* 286 1966
8 Edelmann C M Jr Barnett H L Stark H Boichis H & Rodriguez Soriano J A standardized test of renal concentrating capacity in children *Amer J Dis Child 144* 639 1967
9 *Goldberg M McCurdy Donna K & Ramirez M A Differences* between saline and mannitol diuresis in hydropenic man *J Clin Invest 44* 182 1965
10 *Heyrowski A A new method for the determina*tion of inulin in plasma and urine *Clin Chim Acta 1* 470 1955
11 Kiil F Aukland K & Refsum H E Renal sodium transport and oxygen consumption *Amer J Physiol 201* 511 1961
12 Kleeman C R *Hewitt W L & Guze L B* Pyelonephritis *Medicine 39* 3 1960
13 Lassen N A Munck O & Thaysen J H *Oxygen consumption and sodium reabsorption in* the kidney *Acta Physiol Scand 51* 371 1961
14 Longcope W T & Winkenwerder W L Clini*cal features of the contracted kidney due to* pyelonephritis *Bull Hopkins Hosp 53 255 1933*
15 Lubowitz H Purkerson M L & Bricker N S *Investigation of single nephrons in the chroni*cally diseased (pyelonephritic) kidney of the rat using micropuncture techniques *Nephron 3 73 1966*
16 Michie A J & Michie C R Kidney function in unilateral pyelonephritis I Clinical data *Amer J Med 22 179 1957*
17 Michie A J Michie C R & Ragan M C Kidney function in unilateral pyelonephritis II Physiologic interpretations *Amer J Med 22* 190 1957
18 Polacek E Vocel J Neugebauerova L Seb-kova M & Vecbelova E The osmotic concentrating ability in healthy infants and children *Arch Dis Childh* 40 291 1965
19 Porush J G & Abramson R G The effects of *hypertonic mannitol on renal sodium excretion* in hydropenic man *Clin Sci 29* 475 1965
20 Rieselbach R E Shankel S W Slatopolsky E Lubowitz H & Bricker N S Glucose titration studies in patients with chronic progressive renal disease *J Clin Invest* 46 157 1967
21 Rubin M I Bruck E & Rapoport M Maturation of renal function in childhood clearance studies *J Clin Invest 28* 1144 1949
22 *Schultze R G Slatopolsky E Tall B Walker* W Levy M & Bricker N S The regulation of sodium excretion in uremia a new dimension *in the characterization of* third factor Trans *Ass Amer Physicians* 79 322 1966
23 Schultze R G Shapiro H S & Bricker N S *Studies on the control of sodium excretion in* experimental uremia *J Clin Invest* 48 869 1969
24 Smith H W *The kidney Structure and function in health and disease* Oxford University Press New York 1951
25 Thurau K & Schnermann J Die Natrium*konzentration an den Macula densa Zellen als* regulierender *Faktor* fur das Glomerulumfiltrat (Mikropunktionsversuche) *Klin Wschr 43* 410 1965
26 Thurau K Renal hemodynamics *Amer J Med 36 698 1964*
27 Winberg J Renal concentration capacity during acute nonobstructive urinary tract infections in infancy and early childhood *Acta Paediat Scand 47* 635 1958
28 — Renal function studies in infants and children with acute nonobstructive urinary tract infections *Acta Paediat Scand 48 577 1959*

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After that his mean daily intake of cystine and methionine did not exceed 25 mg and 50 mg per kg body weight respectively. This corresponds with the minimum methionine requirement and a much reduced content of cystine compared to the content in a normal diet.

During the modified dietary treatment he showed a striking improvement. The serum protein rose to normal values and the oedema and skin alterations disappeared. He was very soon in a good clinical condition with a good appetite. In March 1968 he began to speak with single words. In the beginning of April he could walk for a short while without support. He was kept in hospital until April 27th. Since then he has been admitted for a short period every third week. His general condition has continued to be good without relapses. By the termination of the follow up period he was able to run around, he was speaking in sentences and was as a rule gay and happy.

Case 2

Case history. L. R. M. is an elder sister of the patient described above. She was born on June 25th 1960 as the first child of four. Delivery and pregnancy were normal. Her birth weight was 3500 g and her crown heel length 51 cm. She developed normally during the first 9 months of life, but thereafter she began to vomit, she was constipated, refused to eat and failed to thrive. When 18 months old she was admitted to the pediatric department, Municipal Hospital Aarhus, where a metabolic acidosis with alkaline urine, constant proteinuria, hyperaminoaciduria and lowered serum phosphate were demonstrated. There were no clinical signs of rickets and no visible cystine crystals in the corneae. A renal tubular acidosis was suggested. A control admission was planned, but the patient was not brought in. At the time of the admission to the Central Hospital in Holstebro her complaints were thirst and fatigue.

Physical examination. She was admitted on August 4th 1967. She presented as a dwarf with pronounced genu valga due to severe rickets. Her height was 78 cm and weight 12 4 kg. She was pale, with blue eyes, thin blonde hair and remarkably chubby cheeks. She had photophobia and the slitlamp investigation showed cystine crystals in the corneae. She was easily tired, vomited now and then and had polyuria with nycturia 1 2 times each night. Her intelligence was normal. The results of the laboratory investigations on admission are shown in Table 1.

Course. She started the dietary treatment supplemented with cholinechloride 500 mg each day and vitamin D (dihydrotachysterol?) ... [illegible] 0 5 mg each day on the 28th of August 1967. Besides this the classic treatment with vitamin D varying between 18 000 and 36 000 i.u. per day and periodically electrolyte supplements was given. From the very start of the treatment there was great difficulty in getting her to take sufficient amounts of the nourishment prepared from lentils meal.

She was kept in hospital until December 21st 1967 and thereafter she received attention as an in patient for a few days every third week.

Because of increasing difficulty in giving her sufficient calories from the 5th of April the diet was supplemented with buttermilk in quantities varying from 250 to 500 ml per day. Thereafter her daily intake of methionine and cystine did not exceed 35 mg and 20 mg per kg body weight respectively.

The general condition was largely unchanged during the whole investigation period. She was easily tired and occasionally vomited. The serum protein was normal. The haemoglobin was constantly low, the anaemia being normochromic. On four occasions blood transfusions had to be given.

In spite of the above mentioned condition she started normal school in August 1968.

METHODS

The effect of the treatment was estimated from the clinical condition and by repeated laboratory investigations including pyruvate and alphaketoglutarate in the blood, cysteine and cystine excretion in the urine, and amino acid determinations in urine and serum.

Pyruvate and alphaketoglutarate. For the analysis 200 µl of capillary blood treated with 200 µl of 3 5% perchloracid were used. The analysis was performed immediately after the blood had been obtained with reagents from Boehringer (C. F. Boehringer und Soehne GMBH Mannheim, Germany) and was transformed to a macromethod with a measuring volume of 160 µl. A spectrophotometer (Beckman D B) was used for the reading at 366 mµ. The normal values for adults are according to Boehringer 0 14–0 17 mg alphaketoglutarate and 0 4–0 6 mg pyruvate per 100 ml blood.

Cysteine and cystine excretion in the urine. Urine was collected continuously during 24 hours in 5 ml 20 N sulphuric acid. It was kept cool during the collection. Cysteine and cystine in the urine were precipitated as cysteinecuprousmercaptide which was thereafter decomposed. On the addition of 2 6 dichlorchinonechlorimide the liberated cysteine formed a coloured compound that was measured in the spectrophotometer (8). The given values were calculated as cysteine. The normal range for adults with this method is 8 2 48 1 mg per 24 hours (8).

The amino acids in the urine were determined in the department of clinical chemistry, County Hospital of Aalborg, with a Technicon amino acid analyzer. The normal values in Table 3 originate from the normal material of this laboratory of 29 persons in the age group of 3 to 25 years. For further description of the method and the material see Espersen & Puaby (7).

The analysis of *the serum amino acids* was made in Biocentralen, Copenhagen V, on a Technicon amino acid analyzer with an elution time of 10 / hours. The protein was precipitated with sulphosalicylic acid and after centrifugation the supernatant was applied to the column. The normal values in Table 4 are

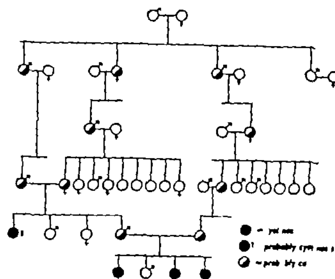


Fig 1 Pedigree The father's oldest sister is indicated as a probable cystinosis patient She died 2 years old after having shown a clinical picture much alike that of our patients

was 54 cm When 14 days old he had chicken pox but otherwise he was healthy and developed normally until the age of 7 months when his appetite became poor he began vomiting developed a remarkable thirst and failed to thrive

Physical examination He was admitted to the medical department Central Hospital in Holstebro on May 10th 1967 His weight was 8500 g and his height 70 cm He was pale with blue eyes thin blonde hair and with strikingly chubby cheeks He had photophobia and examination with the slitlamp revealed cystine crystals in the corneae Radiologically rickets was shown He could sit up on his own and walk a little with support His mental development was normal A pronounced polyuria with a specific gravity of 1001–1002 was found and in spite of a fluid intake of about 5 litres per day he was occasionally dehydrated with flushing cheeks and a moderate fever His appetite was bad and he often vomited The results of the laboratory investigations on admission are shown in Table 1

Course Treatment was started with vitamin D from 0 to 45 000 international units (i u) per day according to the condition potassium citrate/sodium citrate solution corresponding to 25 mEq potassium and 25 mEq sodium per day a combined vitamin preparation (Dolervit®) and oral iron During this regime he continued to have a bad appetite he often vomited and periodically he was slightly dehydrated

From the 5th until the 28th of August the treatment was supplemented with penicillamine one capsule of 150 mg each day Towards the end of this period he developed a severe vitamin D intoxication with serum calcium at 14.4 mg per 100 ml frequent vomitings and a pronounced dehydration which demanded intravenous fluid and electrolyte supplements Otherwise the general condition was unaltered

The dietary treatment was started August 28th 1967 The diet is based on lentils meal as the protein source because this meal is poor in methionine and cystine 100 g of the meal contains 15.5 g protein with 82–145 mg cystine and 136–146 mg methionine Besides lentils meal nutrients poor in or free from protein were given As a supplement he received cholinechloride 500 mg per day and an anabolic steroid metandienon (Dianabol®) 0.3 mg per day The classic treatment was continued

At the beginning of the dietary period he was in a quite good clinical condition but after 2 ½ months he became increasingly debilitated with anorexia vomiting and dehydration He improved only partially after intravenous fluid and electrolyte supplement There were increasing difficulties in giving him sufficient amounts of the diet and during the first fortnight of January 1968 he developed a state of malnutrition with low serum protein the lowest value measured being 4.2 g per 100 ml Simultaneously oedema of the lower extremities developed and the skin on the legs showed redness infiltration and desquamation of large areas which were sharply outlined against the normal skin

On the 14th of January 1968 we stopped the diet and gave cow's milk in free amounts For 10 days the milk was enriched with 0.5% Nestargel® (hydrolyzed animal protein containing free amino acids and with an amino acid nitrogen content of 6 % of the preparation) From January 28th the diet was supplemented only with 500 ml cow's milk per day

Table 1 *Laboratory data at the admission*

	Normal values	Case 1	Case 2
ESR (mm/hour)		11	57
Haemoglobin (g/100 ml)		10.1	9.1
Protein in serum (g/100 ml)	5.6–8.2	7.0	7.4
pH in capillary blood	7.35–7.43	7.30	7.31
Standard bicarbonate (mEq/l)	22.0–25.0	16.6	15.1
Chlorides in serum (mEq/l)	100–107	104	97
Sodium in serum (mEq/l)	136–148	134	133
Potassium in serum (mEq/l)	3.4–4.7	3.0	2.6
Calcium in serum (mg/100 ml)	9–11	10.0	8.1
Phosphate in serum (mg/100 ml)	4.5–7.0	2.4	6.0
Alkaline phosphatase (K A units)	10–35	36.2	56.3
Creatinine in serum (mg/100 ml)	<1.2	0.9	2.3
Diuresis in ml		3620	1900
Protein in urine (g/l)		0.2	1.0
Glucose in urine (g/100 ml)		0.2	0.2
Potassium in urine (mEq/l)		10	23
Sodium in urine (mEq/l)		5	57
Calcium in urine (mg/24 hours)		0	82
Phosphate in urine (mg/24 hours)		320	220
pH in urine		6	7

Table 2 *Pyruvate and alphaketoglutarate in blood before and during dietary treatment*[a]

n numbers of observations

	α ketoglutarate (mg per 100 ml)			R	Pyruvate (mg per 100 ml)			R
	n	Mean	Range	*p*	*n*	Mean	Range	*p*
Case 1 Before treatment	8	0.508	0.30–0.78	35.5 $p<0.01$	8	1.27	1.09–1.86	60 $p>0.1$
During treatment	11	1.038	0.70–1.46		10	1.631	0.91–3.01	
Case 2 Before treatment	4	0.417	0.34–0.45	8 $p<0.01$	4	0.67	0.43–1.03	$0.01<p<0.02$
During treatment	11	0.899	0.43–1.74		10	1.732	0.68–3.11	

[a] For statistical analysis is used the Rank sum (Wilcoxon) test Documenta Geigy Scientific Tables 6th ed pp 124–127

phosphate fluctuated between 3 and 4.4 mg per 100 ml The serum creatinine remained normal In case 2 slightly increasing metabolic acidosis serum phosphate and serum creatin ine were noticed the serum creatinine being 3.3 mg per 100 ml at the end of the follow up period

In case 1 the treatment had no influence on the glucosuria the urinary pH or the diuresis ...ere was slight proteinuria on only a few ...casions The excretion of sodium potassium ...d phosphate did not change during the ...etary treatment From the beginning of Sep...mber 1967 calcium could be detected in the ...ine varying from 0 to 3.7 mg per 100 ml ...case 2 no change at all in the above men...oned values in the urine could be detected ...uring the treatment

Alphaketoglutarate and pyruvate levels in ...he blood during the treatment appear from ...Table 2 It is seen that the concentration of ...lphaketoglutarate rose significantly in both ...atients The blood concentration of pyruvate ...id not rise significantly in case 1 whereas a ...ise was highly probable in case 2

The content of amino acids in urine and serum appears from Tables 3 and 4 The excretion of proline citrulline alanine and valine was pathologically increased in both patients And the excretion of a number of the other

Table 3 *Amino acids in urine (µmoles per 24 hours)*

	Normal values (range)	Lowest and highest values	
		Case 1	Case 2
Taurine	44–1303	0–23	0–217
Aspartic acid	8–158	6–171	22–88
Threonine	43–651	55–555	?–934
Serine	75–942	5–366	?–342
Glutamic acid	5–116	1–670	191–577
Proline	0	1250–6250	606–1793
Citrulline	0	116–537	118–248
Glycine	201–2780	793–1960	1129–1448
α Alanine	67–799	475–2075	865–1409
α Amino-iso-butyric acid	0	0	0
α Amino-n-butyric acid	2 79	9–1562	15–56
Valine	10–12	132–557	165–820
Cystine	17–168	145–443	93–408
Methionine	3–39	0–9	17–94
Isoleucine	3–59	1 17	36–174
Leucine	6–164	9–52	33–397
Tyrosine	26–231	trace–438	16–213
Phenylalanine	15–168	trace 119	54–174
β Amino-iso-butyric acid	14–648	0–39	138–258
Ethanolamine	67–1168	0–107	0
γ-Amino-n-butyric acid	0	0	0
Ornithine	3–92	0–135	56–134
Methylhistidine	33–966	0–trace	0–31
Lysine	28–482	465–1569	141–547
Tryptophane	9–100	0–68	28–33
Histidine	22–1671	367–622	153–422
Arginine	4–44	0–89	40–186

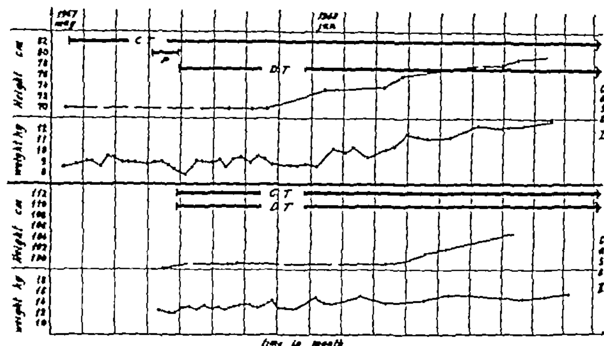


Fig 2 Height and weight in relation to the treatment
C T classic treatment P penicillamine treatment
D T dietary treatment

taken from investigations with the same equipment partly on 30 adults collected from 6 different investigations and partly on 20 children aging from 9 months to 2 years (20)

RESULTS

Classic treatment of case 1 during the first 2 ½ months gave no improvement of the general condition and no gain in weight and height (Fig 2) The rickets was uninfluenced The electrolyte state was only partly improved as metabolic acidosis hypopotassemia, hyponatremia and hypercalcemia were occasionally present and serum phosphate was constantly below 4 mg per 100 ml The great diuresis and the slight glucosuria were unchanged The excretion of potassium in the urine varied from 10 to 20 mEq/l The excretion of sodium in the urine was between 0 and 5 mEq/l until the middle of July 1967 after that time it was usually between 5 and 20 mEq/l Calcium could not be detected in the urine during the first months and the urinary excretion of phosphate did not decrease

Penicillamine treatment During the penicillamine treatment of case 1 there was no demonstrable improvement neither in the general condition, the growth the rickets, nor the el trolyte state The only change was the hyp calcemic crisis at the end of the period

We did not succeed in lowering the bl levels of alphaketoglutarate and pyruvate d ing the penicillamine treatment

Dietary treatment As stated in the c report there was no significant improvement the general condition of case 1 until the c was supplemented with cow s milk But fr that time on there was a striking improvem with gain in weight and height (Fig 2) case 2 the clinical development was not sa factory even though there was a moder gain in height (Fig 2)

The rickets healed in both cases during beginning of January 1968 The necess doses of vitamin D were then tolerated wi out any hypercalcemia

It was not possible to show quantitat changes of the cystine storage in the corn during the treatment in either of the patients

During the modified dietary treatment case 1 a normalization of the serum acid b status and sodium and calcium levels in t serum was obtained But there was occasiona slightly lowered potassium values and seru

ar patients were not exceptions in this respect owards the end of 1967 when case 1 gradually took less of the lentils meal he went into a clear state of malnutrition which might have been due either to a protein depletion or to a state of amino acid imbalance (11) the very low content of cystine in the nourishment and from leading to falling appetite and food intake A combination of both factors may have been responsible

During the whole follow up period case 2 was rather reluctant to take the diet and tube feeding was considered on more than one occasion On the modified diet both patients received more cystine cysteine and methionine per day than advocated by Bauer & Artener (1) but since they thrived best on that diet and as it implied a considerably reduced supply of sulphur containing amino acids we continued that treatment

In the first patient the clinical result was good It is impossible to decide whether this was due to the dietary treatment or to a late manifestation of the conservative treatment or to a combined effect of both treatments We did not succeed in improving the kidney function or reducing the concentrations of pyruvate and alphaketoglutarate with the treatment nor could a quantitative alteration in the cystine storage in the corneae be shown which means that there has been no demonstrable reduction in the cystine content of the body Perhaps this could have been obtained on a more rigid diet On the other hand there is no evidence of a progressive cystine storage during the treatment unless one will interpret the increased concentration in blood of alphaketoglutarate as an indication of increased cystine storage

In case 2 the results as could be expected were not so encouraging although the rickets could be healed and there was some gain in height The treatment could not prevent an increasing glomerular kidney insufficiency and we can only suppose that the development of the uremia had been retarded

The present work allows no ultimate judgment of the effectiveness of the dietary treatment However it seems as if manifest pathological changes will not be influenced by the diet

The spontaneous course of this illness is so serious that any arrangement which will possibly reduce the cystine storage must be advisable We must therefore advise adding to the conservative symptomatic treatment a dietary treatment which has to be started as early as possible in the course of the illness

SUMMARY

Two siblings with cystinosis are presented

Case 1 a 16-month-old boy presented with a severe renal tubular insufficiency Case 2 a 7 year-old girl was a dwarf with both glomerular and tubular renal insufficiency

Case 1 was initially treated with high doses of vitamin D and electrolyte supplements for more than 2 months without significant alteration of the condition Thereafter he was treated for 23 days with 150 mg penicillamine per day again without any significant clinical or biochemical improvement Both patients were then followed through 1 year on treatment with a diet poor in cystine and methionine supplemented with cholinechloride an anabolic steroid high doses of vitamin D electrolytes oral iron and a combined vitamin preparation After some time there was considerable difficulty in giving the patients sufficient amounts of the diet, consequently the diet had to be modified with supplement of cow's milk On this treatment case 1 attained a distinct clinical improvement with healed rickets and normal growth There was no evidence of mobilisation of the stored cystine

Case 2 obtained a healing of the rickets and some gain in height during the treatment but otherwise the general condition was unaltered and she continued to have increasing renal glomerular insufficiency

ACKNOWLEDGEMENTS

The research foundation of the Central Hospital in Holstebro has partly supported this work The lentils

Table 4 *Amino acids in serum (µmoles per l)*

	Normal values (range)		Lowest and highest values	
	Adults	Children	Case 1	Case 2
Taurine	32–138	19–91	68–152	—
Aspartic acid	1–11	0–9	27–75	40–49
Threonine	76–194	33–128	45–168	?–239
Serine	76–164	24–172	84–155	?–152
Glutamic acid	20–90	20–90	131–259	157–179
Proline	103–290	51–185	92–427	278–355
Citrulline			8–56	56–90
Glycine	179–587	56–308	214–329	505–606
α Alanine	213–472	99–313	264–587	408–557
α Amino n butyric acid	10–35	0–17	23–30	12–15
Valine	168–317	57–262	56–333	116–196
Cystine	*70–108*	*0–40*	*0–39*	*16–54*
Methionine	11–30	3–29	6–43	16–31
Isoleucine	40–99	26–94	7–84	29–58
Leucine	78–176	45–155	32–168	62–118
Tyrosine	22–83	11–122	10–56	23–33
Phenylalanine	38–73	23–69	65–94	57–78
Ornithine	30–64	10–107	22–156	73–119
Lysine	105–207	45–144	53–280	134–213
Histidine	32–97	24–112	59–132	83–108
Arginine	40–140	11–65	61–144	125–158

amino acids was occasionally increased The treatment did not normalize this condition

The first investigation of serum amino acids in case 1 was done while he was hypoproteinemic when the concentration of a number of amino acids was rather low In the later investigations the concentration of several of the serum amino acids was a little higher than normal in both patients

The excretion of cystine and cysteine in urine before and during dietary treatment is stated in Table 5 In case 1 it seems as if the concentration in the urine was lower during the treatment In case 2 the concentration in the urine seemed greater during the treatment The fluctuations from the normal adult range

Table 5 *Cystine and cysteine in urine before and during dietary treatment*

Case 1 mg/100 ml				
Before diet	4 8	4 8		
During diet	1 82	0 68	1 02	1 14
Case 2 mg/100 ml				
Before diet	0 94	0 42		
During diet	7 98	4 28	0 64	1 95

are not great, however and the numbers of observations before the dietary treatment are too few to permit a statistical evaluation

DISCUSSION

As long as the aetiology of cystinosis remains unknown uncertainty towards the proper treatment will exist The uncertainty is augmented by the rarity of the disease giving great difficulty in gaining enough experience with new principles of treatment The difficulties in estimating the value of new principles of treatment are in addition increased due to the rather different spontaneous course of the individual cases

Bauer & Antener s (1) combined dietary medical treatment, however seemed to be so great a step forward that we chose to treat our 2 patients according to this principle

It is not possible, on the basis of this investigation to evaluate with certainty the effect of the classic treatment It appears that case 1 was neither clinically nor biochemically improved during the more than 2 months of continuous classic treatment Others (3 12) during several years of continuous therapy with high doses of vitamin D and electrolyte supplement have obtained healed rickets and a normal or improved growth in their cystinosis patients

The 23 days penicillamine treatment of case 1 did not alter his condition in a positive way Even if penicillamine lowers the high blood concentration of pyruvate and alphaketoglutarate in certain cystinosis patients this is certainly not so in all cases (1) and some of the patients develop unpleasant side effects in connection with penicillamine treatment (1 2) It is doubtful whether the hypercalcemic crisis in our patient can be related to the penicillamine treatment

There are often considerable difficulties in treating children with special diets This appears very clearly when a cystinosis patient with anorexia is kept on the rigid and not very palatable cystine and methionine poor diet

r patients were not exceptions in this respect wards the end of 1967 when case 1 gradu y took less of the lentils meal he went into clear state of malnutrition which might have n due either to a protein depletion or to a re of amino acid imbalance (11) the very e content of cystine in the nourishment and man leading to falling appetite and food in ke A combination of both factors may have en responsible

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REFERENCES

1 Bauer B & Antener I Eine wirksame diate tische und medikamentose Cystinosebehandlung *Helv Paediat Acta 21* 19 1966
2 Berger H Antener I Brechbuhler T & Stal der G Zum Problem der Cystinose Beobach tungen uber den Cystinnachweis im Serum und therapeutische Erfahrungen mit Penicillamin und anabolem Hormon *Ann Paediat 202* 465 1964
3 Bickel H Die Entwicklung der biochemischen Lesion bei der Lignac–Fanconischen Krankheit *Helv Paediat Acta 10* 259 1955
4 Bickel H Baar H S Astley R Douglas A A Finch E Harris H Harvey C C Hickmans E M Philpott M G Smallwood W C Smellie J M & Teall C G Cystine storage disease with aminoaciduria and dwarfism *Acta Paediat Scand* Suppl *90* 1 1952
5 Brodehl J Hagge W & Gelissen K Die Veranderungen der Nierenfunktion bei der Cysti nose *Ann Paediat 205* 131 1965
6 Clayton B E & Patrick A D Use of dimer kaprol or penicillamine in the treatment of cystinosis *Lancet II* 909 1961
7 Espersen O & Paaby P Ionbytterkromatogra fisk bestemmelse af aminosyrer i plasma og urin på et normalmateriale *Dan Med Bull* In press
8 Fernandez A A & Henry R J A highly spe cific new method for the determination of cystine and cysteine and its application to urine *Anal Biochem 11* 190 1965
9 Hambraeus L & Broberger O Penicillamine treatment of cystinosis *Acta Paediat Scand 56* 243 1967
10 Hagge W & Brodehl J Die Veranderungen der Nierenfunktion bei der Cystinose *Ann Paediat 205* 442 1965
11 Harper A E Leung P Yoshida A & Rogers Q R Some new thoughts on amino acid im balance *Fed Proc 23* 1087 1964
12 Harrison H E The Fanconi syndrome *J Chron Dis 7* 346 1958
13 Linneweh F Moglichkeiten einer diatetischen Beeinflussung der Cystinose *Klin Wschr 42* 663 1964
14 Patrick A D Deficiencies of SH dependent en zymes in cystinosis *Clin Sci 28* 427 1965
15 Rossier A Caldera R & Odievre M Notions recentes sur la cystinose *Bull Soc Med Hop Paris 114* 1215 1963
16 Scharer K & Antener I Zur Biochemie und Therapie der Cystinose *Ann Paediat* Suppl *1* 1 1964
17 Schneider J A Wong V Bradley K & Seeg miller J E Biochemical comparison of the adult and childhood forms of cystinosis *New Engl J Med 279* 1253 1968
18 Seegmiller J E Friedmann T Harrison H E Wong V & Schneider J A Cystinosis Com bined Clinical Staff Conference at the National Institutes of Health *Ann Intern Med 68* 883 1968
19 Seip M Steen Johnsen J Vellan J E & Gjessing L R Dietary treatment of cystinosis *Acta Paediat Scand 57* 409 1968
20 Soupart P Free amino acids of blood and urine in the human *in* J T Holden (ed) *Amino acid pools* Elsevier Publishing Company Amsterdam London and New York 1962 p 220
21 Weber H & Hagge W Über die erfolgreiche Behandlung der Zystinose mit einem Anabolicum *Arch Kinderheilk 168* 110 1963
22 Worthen H G & Good R A The de Toni Fanconi syndrome with cystinosis *Amer J Dis Child 95* 653 1958

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INTESTINAL DIPEPTIDASES AND DISACCHARIDASES IN CHILDREN WITH MALABSORPTION

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Coeliac disease[1] is the most common protein induced malabsorption syndrome in childhood We know that its clinical symptoms are precipitated by wheat gluten (9) but we do not know why Indirect evidence suggests that the pathogenesis is a lack of a specific peptide hydrolysing enzyme in the intestinal mucosa (5 6 13 23 25 34 35 38) Using a recently developed method for quantitative assay of dipeptidase activities (dipeptide hydrolases EC 3 4 3) in biopsy specimens of the small intestinal mucosa (19 30) we have studied a number of infants and children with coeliac disease before and after dietary treatment

In the acute phase of coeliac disease there is considerable mucosal damage leading to morphological alterations as well as unspecific decrease of for instance the disaccharidase activities These changes are reversible at least partly on treatment with gluten free diet (1 15 18 36 40 41 43) Corresponding secondary changes could be expected to occur with the dipeptidase activities therefore the level of dipeptidase activities in the biopsy specimens studied was compared with the level of disaccharidase activities in the same specimens

In a small group of our patients it appeared that the malabsorption was not gluten induced These patients showed instead a clinical course in many respects similar to that described in cow s milk induced intolerance (8 14 27 44) Therefore these patients were put into a separate group here called "suspected cow s milk intolerance

Some of the results have been presented in a preliminary form (29)

MATERIAL

The patient material consists of 18 infants and children treated at the Paediatric Departments in Lund and Malmo from 1966 to 1969 for diarrhoea vomiting and/or failure to thrive

Coeliac group includes 13 patients (6 boys and 7 girls) aged from 5 months to 13 years (Table 1) All showed a definite clinical improvement (i e their appetite became normal stools normal and they gained weight) on gluten free diet (no wheat rye barley or oats) and their symptoms returned when given unrestricted gluten-containing diet

Suspected cow's milk intolerance" group includes 5 patients (3 boys and 2 girls) aged from 3 to 8 months (Table 2) They showed no or poor clinical improvement on gluten free diet and when by other dietary restrictions (omission of cow's milk) they became symptom free the administration of gluten did not cause any relapse In four of them (14 J J 15 B M L 17 M L 18 R J) elimination of cow's milk was tried and a marked clinical improvement ensued Two of the four (14 J J and 18 R J) were given a provocation test with cow's milk this caused vomiting and diarrhoea These 4 patients were fed human milk which they tolerated well Between the age of 8 and 15 months they began to tolerate cow s milk in small quantities The fifth patient in the suspected cow s milk intolerance group (16 M S)

[1] Also called gluten induced enteropathy coeliac sprue non tropical sprue or idiopathic steatorrhea

Table 1*a* *Coeliac group Clinical and laboratory data (before diet)*

Patient	Age (years)	Gluten free diet (months)	Body weight (S D)	Body height (S D)	Blood xylose (mg/100 ml) 30 min	Blood xylose (mg/100 ml) 60 min	Faecal fat (g/day)	Mucosal morphology: General view of villi	Mucosal morphology: Histological score[a]
1 B B	1/2	0	−1 2	±0	15	25	—[b]	Absent	15
2 M J	1/2	0	−3 4	−1 8	(1)		13	—	—
3 S N	3/4	0	−2 9	±0	(5)[c]		15	Convoluted	14
4 H L	3/4	0	−2 1	−1 4	16	24	1	Absent	17
5 J U	3/4	0	−2 4	−1 7	2	4	—	Absent	16
6 A L	3/4	0	−3 7	−1 2	—		12	—	—
7 M L	1	0	−2 8	−2 2	(10)		11	Absent	17
8 Ch Z	1 1/4	0	−3 1	−3	24	30	5	Absent	18
9 V N	1 1/2	0	−2 5	−2 5	14	17	3	Absent	16
10 T E	1 3/4	0	−2 1	−2 4	14	19	5	Absent	17
11 S M	2	0	−2 3	−2 7	13	18	12	—	—
12 R S	7 1/2	0	−1	±0	18	27	4	Absent	18
13 L B P	13	0	−2 5	−2 5	2	3	37	Short broad	12
Normal values					>15	>25	<4		<4

[a] Histological score with respect to villous structure changes of surface epithelium and infiltration with inflammatory cells normal <4 points slight damage −9 points grave damage >14 points
[b] — = not examined
Urinary xylose excretion normal values >15

received gluten free diet containing cow's milk from age 6 to 25 months During this period his condition improved very slowly but at 25 months his xylose absorption and his intestinal mucosa had become normal From 27 months he tolerated an unrestricted diet and when his intestinal mucosa was studied after 9 months without any dietary restrictions it was found to be still normal

Further data on some of the patients in this group have been presented (2)

Control group Because of the difficulties involved in obtaining a strictly normal control material we have used a control group consisting of 12 infants and children (aged 2 months to 14 years) and 37 adults (3) with symptoms from the gastro intestinal

Table 1*b* *Coeliac group Clinical and laboratory data (after dietary treatment)*

Patient	Age (years)	Gluten free diet (months)	Body weight (S D)	Body height (S D)	Blood xylose (mg/100 ml) 30 min	Blood xylose (mg/100 ml) 60 min	Faecal fat (g/day)	Mucosal morphology: General view of villi	Mucosal morphology: Histological score[a]
1 B B	Not studied								
2 M J	3	29[d]	+1 2	+1 8	15	16	2	Short broad	11
3 S N	2	16	+0 7	+0 1	36	88	3	Short broad	11
4 H L	Not studied								
5 J U	2 1/4	17	−0 2	−0 4	33	32	—[b]	Short broad	9
6 A L	2 1/2	0[e]	−1 2	±0	29	42	7	Absent	17
7 M L	1 1/4	4	−1 7	−2 1	(12)		1	Broad leaves	11
8 Ch Z	1 3/4	8	−0 7	−1 4	41	59	—	Finger leaves	7
9 V N	2	7	−0 5	−2 5	29	42	3	Convoluted	13
10 T E	2 1/2	10	−1 2	−2 0	28	16	4	Short broad	11
11 S M	3	12	−1 9	−2 6	41	45	2	Finger leaves	7
12 R S	8	9	−0 4	+1	40	49	2	Convoluted	14
13 L B P	14	9	−0 8	−1 6	22	50	—	Finger leaves	7
Normal values					>15	>25	4		<4

[b] [c] For explanation see Table 1*a*
[d] Not strictly gluten free diet all the time
Gluten free diet for 15 months gluten-containing diet the last 4 months before biopsy
The biopsy has therefore been included in the untreated group in Figs 1 and 2

e 2a Suspected cow's milk intolerance group Clinical and laboratory data (before diet)

		Diet (months)				Blood xylose (mg/100 ml)			Mucosal morphology	
nt	Age (months)	Cow's milk free	Gluten free	Body weight (S D)	Body height (S D)	30 min	60 min	Faecal fat (g/day)	General view of villi	Histol score[a]
J	2	0	0	−2.3	−1.0	—[b]	—	7	—	—
M I	5	0	0	−2.9	−1.2	12	18	6	Convoluted	12
S	6	0	0	−2.3	−2.4	15	16	5	Absent	18
L	7	0	2	−2.2	−3.0	9	18	24	Absent	18
J	3	0	0	−1.0	±0	(10)		—	—	—
mal values						>13	>25	<4		<4

For explanation see Table 1a

, but with histologically normal mucosa obtained iopsy As there was no significant difference between mean values for enzyme activity in the children and in the adults the control material was combined into a single group

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Morphological examination

The biopsy specimen was oriented lumenal side upwards on a fine plastic net and then fixed in Bouin solution or in formaldehyde solution within short after fixation in Bouin solution The fixed specimens were studied in a dissection microscope and photographed The same technician prepared the specimens and cut them serially in 5–6 μm sections taking care to keep the cutting plane vertical to the mucosal surface Alternative slides were stained with haematoxylin and erythrosin, with van Gieson stain and with PAS stain according to McManus The best orientated sections from the central part of the biopsy specimens were carefully studied in the light microscope and coded with respect to villous structure changes of the surface epithelium and infiltration with plasma cells and neutrophil granulocytes (Berg et al —in preparation) (See Table 1 a)

Assay of dipeptidase activities

The mucosal specimen was homogenized and assayed for dipeptidase activity as described earlier (19 30) Gly glu NH (glycyl L glutamine) was obtained from Cyclo Chem Co (Los Angeles Calif) A 0.04 M aqueous solution was used pH optimum was 7.7 (Lindberg unpublished) The other substrates were the same as used earlier (19 20 21) No metal ion activator was added (One unit of dipeptidase is the activity hydrolysing 1 μmole of dipeptide per min at 40 C)

Assay of disaccharidase activities

After homogenization with a glass pestle the disaccharidase activities were analysed by the method earlier described (7) (One unit of disaccharidase activity hydrolyses 1 μmole of disaccharide per min at 37 C)

Assay of protein

The method of Lowry et al (31) was used In accord with earlier publications the disaccharidase activities were calculated as unit per g protein and the dipeptidase activities as units per mg nitrogen The nitrogen content was calculated as 17% of the protein content

The following abbreviations are used ala glu (L alanyl L glutamic acid) ala pro (L alanyl L proline) gly leu (glycyl L-leucine) gly val (glycyl L valine) glu pro (L-glutamyl L proline) glu val (L glutamyl L valine) and val glu (L valyl L glutamic acid)

Table 1*a* *Coeliac group Clinical and laboratory data (before diet)*

Patient	Age (years)	Gluten free diet (months)	Body weight (S D)	Body height (S D)	Blood xylose (mg/100 ml) 30 min	60 min	Faecal fat (g/day)	Mucosal morphology General view of villi	Histological score[a]
1 B B	1/2	0	−1 2	±0	15	25	—[b]	Absent	15
2 M J	1/2	0	−3 4	−1 8	(1)[c]		13	—	—
3 S N	3/4	0	−2 9	±0	(5)		15	Convoluted	14
4 H L	3/4	0	−2 1	−1 4	16	24	1	Absent	17
5 J U	3/4	0	−2 4	−1 7	2	4	—	Absent	16
6 A L	3/4	0	−3 7	−1 2	—		12	—	—
7 M L	1	0	−2 8	−2 2	(10)[c]		11	Absent	17
8 Ch Z	1 1/4	0	−3 1	−3	24	30	5	Absent	18
9 V N	1 1/2	0	−2 5	−2 5	14	17	3	Absent	16
10 T E	1 3/4	0	−2 1	−2 4	14	19	5	Absent	17
11 S M	2	0	−2 3	−2 7	13	18	12	—	—
12 R S	7 1/2	0	−1	±0	18	27	4	Absent	18
13 L B P	13	0	−2 5	−2 5	2	3	37	Short broad	12
Normal values					>15	>25	<4		<4

[a] Histological score with respect to villous structure changes of surface epithelium and infiltration with inflammatory cells normal <4 points slight damage = 9 points grave damage >14 points
[b] — = not examined
[c] Urinary xylose excretion normal values >15

received gluten free diet containing cow s milk from age 6 to 25 months During this period his condition improved very slowly but at 25 months his xylose absorption and his intestinal mucosa had become normal From 27 months he tolerated an unrestricted diet and when his intestinal mucosa was studied after 9 months without any dietary restrictions it was found to be still normal

Further data on some of the patients in this group have been presented (2)

Control group Because of the difficulties involved in obtaining a strictly normal control material we have used a control group consisting of 12 infants and children (aged 2 months to 14 years) and 37 adults (3) with symptoms from the gastro-intestinal

Table 1*b* *Coeliac group Clinical and laboratory data (after dietary treatment)*

Patient	Age (years)	Gluten free diet (months)	Body weight (S D)	Body height (S D)	Blood xylose (mg/100 ml) 30 min	60 min	Faecal fat (g/day)	Mucosal morphology General view of villi	Histological score[a]
1 B B	Not studied								
2 M J	3	29[d]	+1 2	+1 8	15	16	2	Short broad	11
3 S N	2	16	+0 7	+0 1	36	88	3	Short broad	11
4 H L	Not studied								
5 J U	2 1/4	17	−0 2	−0 4	33	32	—[b]	Short broad	9
6 A L	2 1/2	0[e]	−1 2	±0	29	42	7	Absent	17
7 M L	1 1/4	4	−1 7	−2 1	(12)		1	Broad leaves	11
8 Ch Z	1 3/4	8	−0 7	−1 4	41	59	—	Finger leaves	7
9 V N	2	7	−0 5	−2 5	29	42	3	Convoluted	13
10 T E	2 1/2	10	−1 2	−2 0	28	16	4	Short broad	11
11 S M	3	12	−1 9	−2 6	41	45	2	Finger leaves	7
12 R S	8	9	−0 4	+1	40	49	2	Convoluted	14
13 L B P	14	9	−0 8	−1 6	22	50	—	Finger leaves	7
Normal values					15	>25	<4		<4

[a] [b] For explanation see Table 1*a*
[d] Not strictly gluten free diet all the time
[e] Gluten free diet for 15 months gluten-containing diet the last 4 months before biopsy The biopsy has therefore been included in the untreated group in Figs 1 and 2

Table 2e *Suspected cow's milk intolerance group Clinical and laboratory data (before diet)*

		Diet (months)				Blood xylose (mg/100 ml)			Mucosal morphology	
Patient	Age (months)	Cow's milk free	Gluten free	Body weight (S D)	Body height (S D)	30 min	60 min	Faecal fat (g/day)	General view of villi	Histol score[a]
H J J	2	0	0	−2.3	−1.0	—[b]	—	7	—	—
B S H L	5	0	0	−2.9	−1.2	12	18	6	Convoluted	12
K N S	6	0	0	−2.3	−2.4	15	16	5	Absent	18
P M L	7	0	2	−2.2	−3.0	9	18	24	Absent	18
H R J	7	0	0	−1.0	±0	(10)		—	—	—
Normal values						>15	>25	<4		<4

[a] For explanation see Table 1e

[illegible], but with histologically normal mucosa obtained at biopsy. As there was no significant difference between [illegible] for enzyme activity in the children and in the adults, the control material was combined into a single group.

METHODS

Laboratory tests

The possibility of pancreatic disease as a cause of malabsorption in our patients was excluded by determination of chymotrypsin in faeces (16) and sweat test. For determination of faecal fat excretion the patient was fed a diet with known fat content and specimens of the stools were analyzed for 3 days (??). The absorption of D xylose was studied after a dose of 15 g m[2] body surface. Blood xylose and/or urinary xylose excretion were determined (37 32). Lactose and glucose-galactose tolerance tests were performed with a dose of 2 g kg body weight.

Biopsy technique

Biopsy of the small intestinal mucosa in all patients was taken at the duodeno-jejunal flexure under fluoroscopic control. (We use a hydraulic multiple biopsy capsule, Meeuwisse et al, to be published.) We took at least three specimens each weighing about 5 mg. One was used for morphological examination, one for assay of dipeptidase activity and one for disaccharidase activity. The pieces to be used for enzyme assay were enveloped in parafilm and stored at −20 C until analysis. Stored thus the enzyme activities studied are stable for months.

Morphological examination

The biopsy specimen was oriented luminal side upwards on a fine plate, [illegible] and then fixed in Bouin solution or in formaldehyde solution with short after fixation in Bouin solution. The fixed specimens were studied in a dissection microscope and photographed. The tissue technician prepared the specimens and cut them [illegible] sections taking care to keep the cutting plane vertical to the mucosal surface. Alternative slides were stained with haematoxylin and erythrosin, with van Gieson stain and with PAS stain according to McManus. The best orientated sections from the central part of the biopsy specimens were carefully studied in the light microscope and coded with respect to villous structure, changes of the surface epithelium, and infiltration with plasma cells and neutrophil granulocytes (Berg et al — in preparation) (See Table 1 e).

Assay of dipeptidase activities

The mucosal specimen was homogenized and assayed for dipeptidase activity as described earlier (19 20). Gly glu NH_2 (glycyl L glutamine) was obtained from Cyclo Chem Co (Los Angeles Calif). A 0.04 M aqueous solution was used, pH optimum was 7.7 (Lindberg unpublished). The other substrates were the same as used earlier (19 20 21). No metal ion activator was added. (One unit of dipeptidase is the activity hydrolyzing 1 μmole of dipeptide per min at 40 C.)

Assay of disaccharidase activities

After homogenization with a glass pestle the disaccharidase activities were analysed by the method earlier described (7). (One unit of disaccharidase activity hydrolyzes 1 μmole of disaccharide per min at 37 C.)

Assay of protein

The method of Lowry et al (31) was used. In accord with earlier publications the disaccharidase activities were calculated as unit per g protein and the dipeptidase activities as units per mg nitrogen. The nitrogen content was calculated as 17 [illegible] of the protein content.

The following abbreviations are used: al glu (L alanyl L glutamic acid), ala pro (L alanyl L proline), gly leu (glycyl L leucine), gly val (glycyl L valine), glu pro (L glutamyl L proline), glu val (L glutamyl L valine) and val glu (L valyl L glutamic acid).

Table 2*b* *Suspected cow's milk intolerance group Clinical and laboratory data (after dietary treatment)*

Patient	Age (years)	Diet (months) Cow's milk free	Gluten free	Body weight (S D)	Body height (S D)	Blood xylose (mg/100 ml) 30 min	60 min	Faecal fat (g/day)	Mucosal morphology General view of villi	Histol score[a]
14 J J	1/2	5	5	−0 9	−1 8	(20)[c]		1	Finger leaves	7
15 B M L	1 1/4	0[d]	10	−1 4	−1 3	10	18	—[b]	Partly convoluted partly short broad	10
16 N S	2	0	19	−1 4	−2 5	35	47	—	Finger leaves	5
17 M L	1	0[e]	6	−0 5	−0 6	11	21	—	Convoluted	11
18 R J	1 1/4	7	7	−0 2	−1 5	(10)		1	Finger leaves	9
Normal values						15	>25	<4		<4

[a, b, c] For explanation see Table 1*a*
[d] Cow's milk containing diet from 10 months of age
[e] Cow's milk containing diet from 6 months of age
Comments 14 Tolerating normal diet from 8 months 2 years observation 15 Tolerating normal diet from 1 y 8 mo 2 years observation 16 Tolerating normal diet from 2 y 3 mo 1 year's observation 18 Tolerating normal diet from 1 y 3 mo 1 year's observation

RESULTS

Some of the clinical and laboratory findings in the two patient groups are summarized in Tables 1 and 2 Tables 1 *a* and 2 *a* show the data before treatment and Tables 1 *b* and 2 *b* the data after Body weight and height were calculated as standard deviations (S D) from the average of Swedish children (4 24)

Before dietary treatment xylose absorption was decreased in 9 of the 13 patients in the coeliac group (Table 1 *a*) and in 4 of the 5 patients in the suspected cow's milk intolerance group (Table 2 *a*) Faecal fat excretion was increased in 8 (out of 11 studied) in the coeliac group and in 4 of the patients in the other group The morphological examination of the biopsy specimens revealed a flat mucosa of coeliac pattern (cf Rubin & Dobbins (39)) in all the patients of the coeliac group and in 3 of those in the other group In the two remaining patients of the suspected cow's milk intolerance group, biopsy was not performed before treatment

After 4–29 months of gluten free diet treatment 10 of the patients in the *coeliac group* were again examined (see Table 1 *b*) All had gained weight and showed improved xylose absorption The faecal fat excretion was measured in 8 of them, it was normal in 7 The morphology of the intestinal mucosa showed a more or less marked improvement (Table 1*b*)

Fig 1 shows the dipeptidase activities in the group before and after treatment compared with the range of the control group and Fig the corresponding values for the disaccharidase activities The enzyme activities showed a skew distribution in a linear diagram, but a normal distribution when the corresponding logs were used (3) The enzyme activities were therefore plotted on a log scale Before dietary treatment the dipeptidase activities in most of the coeliac patients were much lower than in the controls The decrease was most pronounced for the ala pro dipeptidase activity After treatment there was a marked increase in dipeptidase activity thus in most cases values within or close to the range of controls were obtained Also at this stage in relation to the control group the ala pro dipeptidase activity was lower than any of the other dipeptidase activities None the less there was an increase also in the ala pro dipeptidase activity during treatment (Fig 1) The glu val dipeptidase activity also behaved somewhat strangely in 3 patients it decreased during treatment in the other it increased

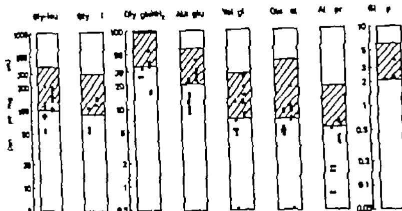


Fig 1 Dipeptidase activities in small intestinal biopsy specimens in the coeliac group ● Untreated ○ after treatment with gluten free diet ▧ controls range

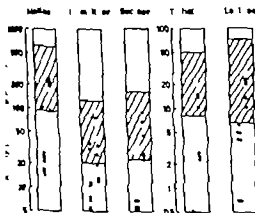


Fig 2 Disaccharidase activities in small intestinal biopsy specimens in the coeliac group For explanation of symbols see Fig 1

The disaccharidase activities were low at the first examination (Fig 2) The decrease in enzyme activity calculated as a percentage of the mean value for the control group was still more marked than for the dipeptidases (Table 3) The lactase and trehalase activities were proportionally somewhat more markedly decreased than the other disaccharidases After treatment there was a considerable increase in all the disaccharidase activities (Fig 2 Table 3)

All 5 patients in the *suspected cow's milk intolerance group* were examined after 5–19 months of dietary treatment (Table 2 *b*) All

Table 3 Specific activity of intestinal dipeptidases and disaccharidases in per cent of the mean values found in controls

Enzyme activity	Coeliac disease		"Suspected cow's milk intolerance"		
	Untreated ($n = 12$)	Treated ($n = 10$)	Untreated ($n = 3$)	Treated ($n = 5$)[a]	Controls ($n = 49$)
Gly leu dipeptidase	45	76	35	64	100 (190)
Gly val dipeptidase	57	60	33	54	100 (172)
Gly gluNH dipeptidase	37	50			100 (62)
Ala glu dipeptidase	38	65	37	56	100 (36 3)
Val glu dipeptidase	31	87	49	52	100 (16 2)
Glu val dipeptidase	27	37	22	48	100 (18 9)
Ala pro dipeptidase	17	40	25	42	100 (10 8)
Glu pro dipeptidase	31	50	31	47	100 (3 6)
Maltase	16	50	12	50	100 (214)
Isomaltase	23	72	17	72	100 (57.2)
Sucrase	16	62	20	57	100 (60 2)
Trehalase	10	43	6	77	100 (22.1)
Lactase	10	40	5	46	100 (29 7)

Figures within parentheses are units mg nitrogen (dipeptidases) and units/g protein (disaccharidases) respectively in the control group (mean value)
[a] For dipeptidases ...

Table 2*b* "Suspected cow s milk intolerance group Clinical and laboratory data (after dietary treatment)

		Diet (months)				Blood xylose (mg/100 ml)			Mucosal morphology	
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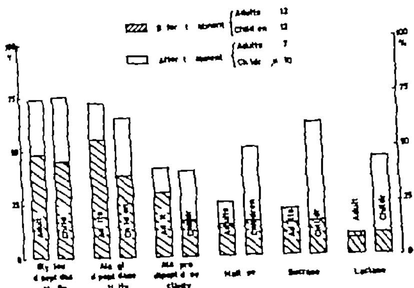


Fig. 5. Mean values of specific activities of intestinal dipeptidases and disaccharidases in coeliac disease in children and adults (3) expressed as per cent of the mean value for the corresponding activity in the control group.

seem to be secondary in nature. The decrease was seen with all the dipeptides and when calculated as percentages of the mean value of the control group the dipeptidase activities in our coeliac patients were not decreased to the same extent as were the disaccharidases (Table 3). The ala pro dipeptidase activity was apparently more strongly affected than the other dipeptidases with only 17 % of the "normal" activity remaining before treatment. None the less four of the five disaccharidase activities studied were more strongly depressed than the ala pro dipeptidase; furthermore during dietary treatment, the ala pro dipeptidase activity increased up to 40 % of the "normal" value, which would not be expected if this activity was the expression of an enzyme deficiency in coeliac disease. The glu val dipeptidase activity decreased in 3 cases during treatment but in the other 7 it increased parallel with the other enzyme activities. The unspecific decrease in the dipeptidase activities in the intestinal mucosa from patients with coeliac disease agrees with the results of several authors who have used different substrates and techniques (10, 12, 17, 35) but disagrees with the results of Messer et al. (33) who could not find any certain difference between the dipeptide splitting capacity in untreated coeliac and normal mucosa. To our knowledge 24 dipeptides have so far been tested by different authors in attempts to find a specific enzyme defect in coeliac disease.

There is a difference between the coeliac children studied here and coeliac adults (3) concerning the behaviour of the dipeptidases and the disaccharidases (Fig. 5). In adults and children the dipeptidase activities improved to the same level. The rise of the disaccharidases however was much slower in the adults than in the children. The mean lactase activity in the adults failed to improve. Both in children and adults the change in trehalase activity closely followed that of lactase although we know that these two activities are exerted by different enzymes.

The other patient group in our material is not so well defined as the coeliac group, but we have good reasons for believing that those patients suffered from cow's milk intolerance. In some of them provocation tests with cow's milk were performed with positive result. Others were initially supposed to suffer from coeliac disease and were treated with gluten free diet, but after varying periods the diagnosis had to be revised and a diet with neither cow's milk nor gluten was introduced. The morphological changes in the mucosal biopsy

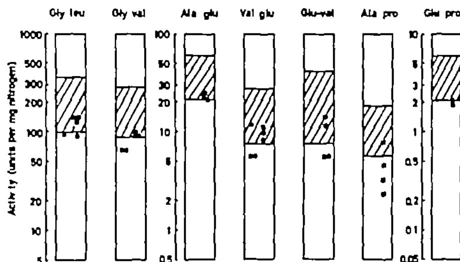


Fig 3 Dipeptidase activities in small intestinal biopsy specimens in the suspected cow's milk intolerance group ■ Untreated □ after dietary treatment, for details see text and Table 2 ▨ controls range

were given gluten free diet but cow's milk was excluded in only 2 of them Furthermore only 3 of the patients had a biopsy taken before treatment, and although all 5 were biopsied after treatment, dipeptidase activities were measured in only 4 of them Thus the data for this small group are rather incomplete All gained weight during the dietary treatment, and the steatorrhea had disappeared, but in only two of them was the xylose absorption normal (Table 2 *b*) Histologically the mucosa showed changes similar to those in the coeliac group and seemed to undergo a similar normalization during dietary treatment The dipeptidase and disaccharidase activities were low and increased during dietary treatment

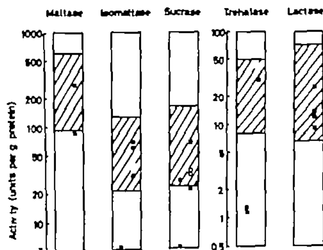


Fig 4 Disaccharidase activities in small intestinal biopsy specimens in the suspected cow's milk intolerance group For explanation of symbols see Fig 3

(Fig 3–4 Table 3) We could not find any morphological or enzymological differences between this group and the coeliac group It has been reported that some patients with cow's milk intolerance develop a transient intolerance for gluten (14 44) but this was not observed in our patients

DISCUSSION

All in our coeliac disease group seem to suffer from the classical form of the disease with a typical history characteristic clinical and laboratory findings and rapid improvement on gluten free diet In 5 of our patients, we demonstrated a relapse on administration of gluten after the intestinal mucosa had first become normal (the criterion recommended by the European Society for Paediatric Gastroenterology for a strict diagnosis of coeliac disease) The others in this group have not yet been followed sufficiently long for this test to be performed, but all relapsed clinically when unrestricted diet was given after at least 6 months of gluten free diet

The low disaccharidase activities found in the coeliac group and the increase in these activities on dietary treatment agree with earlier reports (1 15 18 36 40 41 43) These changes apparently occur parallel with the morphological changes in the mucosa

The decreased dipeptidase activities in coeliac patients found with the substrates we used

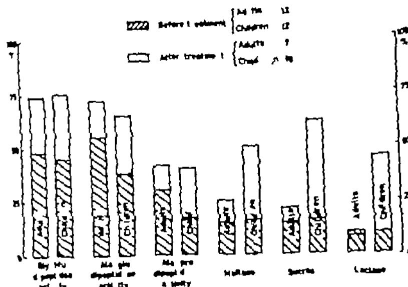


Fig 5 Mean values of specific activities of intestinal dipeptidases and disaccharidases in coeliac disease in children and adults (3) expressed as per cent of the mean value for the corresponding activity in the control group

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There is a difference between the coeliac children studied here and coeliac adults (3) concerning the behaviour of the dipeptidases and the disaccharidases (Fig 5) In adults and children the dipeptidase activities *improved* to the same level The rise of the disaccharidases however was much slower in the adults than in the children The mean lactase activity in the adults failed to improve Both in children and adults the change in trehalase activity closely followed that of lactase although we know that these two activities are exerted by different enzymes

The other patient group in our material is not so well defined as the coeliac group but we have good reasons for believing that those patients suffered from cow's milk intolerance In some of them provocation tests with cow's milk were performed with positive result Others were initially supposed to suffer from coeliac disease and were treated with gluten free diet, but after varying periods the diagnosis had to be revised and a diet with neither cow's milk nor gluten was introduced The morphological changes in the mucosal biopsy

from these patients were very similar to those in the coeliac group The only other condition with such changes recognized in Scandinavian paediatric practice and affecting the proximal part of the gut is cow's milk intolerance (26 44) *This intolerance* disappeared at about one year of age in our patients which agrees with earlier reports (14 44)

In both coeliac disease and cow's milk intolerance, damage of the intestinal mucosa seems to be provoked by a dietary protein Thus for the cow's milk intolerance, as well as for the coeliac disease, it is possible that the absence of some enzyme necessary for protein or peptide hydrolysis is responsible for the disease Nor in this group however did our dipeptide substrates provide any clue to the specificity of such a hypothetical enzyme The dipeptidase and disaccharidase activities tested were all low in the untreated phase of the disease The findings of the disaccharidases agree with those reported by Silver & Douglas (42) This decrease of enzyme activity was reversible on dietary treatment In fact, the similarity in the changes in the mucosal enzyme activities in the suspected cow's milk intolerance group to those in the coeliac group was as striking as the morphological changes in the two groups

SUMMARY

Intestinal enzyme activities (dipeptidases and disaccharidases) and mucosal morphology were studied in two groups of paediatric patients one coeliac group ($n = 13$) and one suspected cow's milk intolerance group ($n = 5$) The patients were studied before and after dietary treatment 49 children and adults with histologically normal intestinal mucosa served as a control group

Both groups of patients showed a marked secondary decrease in the dipeptidase and the disaccharidase activities The decrease was more pronounced for the disaccharidase than for the dipeptidase activities and was reversible on dietary treatment Among the disaccharidases the lactase and trehalase activities were most strongly depressed, in the coeliac group the ala pro dipeptidase activity was more strongly affected than the other dipeptidases However as also the ala pro dipeptidase activity increased on dietary treatment, this does not provide any clue to the specificity of a hypothetical enzyme whose absence could be responsible for the disease

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REFERENCES

1 Arthur A B Intestinal disaccharidase deficiency in children with coeliac disease *Arch Dis Child 41* 519 1966
2 Berg N O Dahlqvist, A Lindberg T Norwise G W & Akerman M Small intestinal mucosal structure disaccharidases and dipeptidases in different types of malabsorption in childhood A longitudinal study International Conference on Coeliac Disease London 1969
3 Berg N O Dahlqvist A Lindberg T & Norden A Intestinal dipeptidases and disaccharidases in celiac disease in adults *Gastroenterology In press 1970*
4 Broman B Dahlberg G & Lichtenstein A Height and weight during growth *Acta Paediat Scand 30* 1 1942
5 Bronstein H D Haeffner L J & Kowlessar O D Enzymatic digestion of gliadin The effect of the resultant peptides in adult celiac disease *Clin Chim Acta 14* 141 1966
6 Crabbe P Sprue non tropical étiologie et pathogénie *Acta Gastroent Belg 27* 8 1964
7 Dahlqvist A Assay of intestinal disaccharidases *Anal Biochem 22* 99 1968
8 Davidson M Burnstine R C Kugler M M & Bauer C H Malabsorption defect induced by ingestion of beta lactoglobulin *J Pediat 66* 545 1965
9 Dicke W K Weijers H A & Kamer J H van de Coeliac disease II The presence in wheat of a factor having a deleterious effect in cases of coeliac disease *Acta Paediat Scand 42* 34 1953
10 Dolly J O & Fottrell P F Multiple forms of dipeptidases in normal human intestinal mucosa

and in mucosa from children with coeliac disease *Clin Chim Acta* 26 535 1969

11 Douglas A P & Booth C C Jejunal mucosal digestion of gluten peptides in adult coeliac disease *Lancet* 2 491 1968

12 Douglas A P & Peters T J Studies on intestinal peptidases with special reference to coeliac disease International Conference on Coeliac Disease London 1969

13 Frazer A G Discussion on some problems of steatorrhea and reduced stature *Proc Roy Soc Med* 49 1009 1956

14 Fällström S P Winberg J & Andersen H J Cow's milk induced malabsorption as a precursor of gluten intolerance *Acta Paediat Scand* 54 101 1965

15 Hamilton J F Lynch M J & Reilly B J Active coeliac disease in childhood Clinical and laboratory findings of forty two cases *Quart J Med* 38 135 1969

16 Haverback B J Dyce B J Gutentag P J & Montgomery D W Measurement of trypsin and chymotrypsin in stool A diagnostic test for pancreatic exocrine insufficiency *Gastroenterology* 44 588 1963

17 Heizer W D & Laster L Peptide hydrolase activities of the mucosa of human small intestine *J Clin Invest* 48 210 1969

18 Jos J Frézal J Rey J & Lamy M Étude histochimique de la muqueuse duodéno-jéjunale dans la maladie coeliaque *Pediat Res* 1 27 1967

19 Josefsson L & Lindberg T Intestinal dipeptidases I Spectrophotometric determination and characterization of dipeptidase activity in pig intestinal mucosa *Biochim Biophys Acta* 105 149 1965

20 — Intestinal dipeptidases III Characterization and determination of dipeptidase activity in adult rat intestinal mucosa *Acta Physiol Scand* 66 410 1966

21 — Intestinal dipeptidases IX Studies on dipeptidases of human intestinal mucosa *Acta Chem Scand* 21 1965 1967

22 Kamer van de J H ten Bokkel Huinink H & Weyers H A Rapid method for the determination of fat in the feces *J Biol Chem* 177 347 1949

23 Kamer van de J H & Weyers H A The diagnosis of protein malabsorption Proceedings Europ Soc Pediat Gastroenterol Interlaken 1969

24 Karlberg P Engström I Lichtenstein H & Svennberg I The development of children in a Swedish urban community A prospective longitudinal study III Physical growth during the first three years of life *Acta Paediat Scand* Suppl 187 48 1968

25 Krainick H G & Mohn G Weitere Untersuchungen über den schädlichen Weizenmehleffekt bei der Cöliakie 2 Die Wirkung der enzymatischen Abbauprodukte des Gliadin *Helv Paediat Acta* 14 124 1959

26 Kuitunen P Visakorpi J K & Hallman N Histopathology of duodenal mucosa in malabsorption syndrome induced by cow's milk *Ann Paediat* 205 54 1965

27 Lamy M Nezelof C Jos J Frézal J & Rey J La biopsie de la muqueuse intestinale d'une des syndromes de malabsorption *Presse Med* 71 1267 1963

28 Lindberg T Intestinal dipeptidases Dipeptidase activity in the mucosa of the gastrointestinal tract of the adult human *Acta Physiol Scand* 66 437 1966

29 — Dipeptidase activity in small intestinal mucosa in patients with gluten induced enteropathy *Acta Paediat Scand* Suppl 177 23 1967

30 Lindberg T Nordén A & Josefsson L Intestinal dipeptidases Dipeptidase activities in small intestinal biopsy specimens from a clinical material *Scand J Gastroent* 3 177 1968

31 Lowry O H Rosebrough N J Farr A L & Randall R J Protein measurement with the Folin phenol reagent *J Biol Chem* 193 262 1951

32 Mecrowne G W & Dano G The xylose absorption test in infancy *Acta Paediat Scand* 54 33 1965

33 Messer M Anderson Ch M & Townley R R W Peptidase activity of biopsies of the duodenal mucosa of children with and without coeliac disease *Clin Chim Acta* 6 768 1961

34 Messer M Anderson Ch M & Hubbard L Studies on the mechanism of destruction of the toxic action of wheat gluten in coeliac disease by crude papain *Gut* 5 295 1964

35 Pittman F E & Pollitt R J Studies of jejunal mucosal digestion of peptic tryptic digests of wheat protein in coeliac disease *Gut* 7 368 1966

36 Plotkin G R & Isselbacher K J Secondary disaccharidase deficiency in adult celiac disease (non tropical sprue) and other malabsorption states *New Eng J Med* 271 1033 1964

37 Roe E W & Rice J H A photometric method for the determination of free pentoses in animal tissues *J Biol Chem* 173 507 1948

38 Roon J H van & Haex A J Ch Clinical and biochemical analysis of gluten toxicity No II *Gastroenterologia* 94 227 1960

39 Rubin C E & Dobbins W O III Peroral biopsy of the small intestine A review of its diagnostic usefulness *Gastroenterology* 49 676 1965

40 Schmerling D H Auricchio S Rubino A Hadorn B & Prader A Der sekundäre Mangel an intestinaler Disaccharidaseaktivität bei der Coeliakie Quantitative Bestimmung der Enzymaktivität und klinische Beurteilung *Helv Paediat Acta* 19 507 1964

41 Sheldon W & Tempany E Small intestine peroral biopsy in coeliac children *Gut* 7 481 1966

42 Silver H & Douglas D M Milk intolerance in infancy *Arch Dis Child 43* 17 1968
43 Welsh J D Zschiesche O M Anderson J & Walker A Intestinal disaccharidase activity in celiac sprue (gluten sensitive enteropathy) *Arch Intern Med 123* 33 1969
44 Visakorpi J K & Immonen P Intolerance to cow s milk and wheat gluten in the primary malabsorption syndrome in infancy *Acta Paediat Scand 56* 49 1967

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THE LOWE SYNDROME

Observations on the Amino Acid Metabolism in a 2 year-old Affected Boy

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Since the original description by Lowe et al (9) in 1952 a substantial number of reports on the oculo-cerebro-renal syndrome have been published and an extensive review of the clinical and laboratory findings in this disease based on 57 cases from the literature was recently offered by Abbassi et al (1) In despite of the fact that marked aminoaciduria is one of the salient characteristics of the disease rather few quantitative studies of amino acid concentrations in blood, urine and cerebro-spinal fluid are available

The present report concerns a 2 year-old boy in whom a diagnosis of oculo-cerebro-renal syndrome was made at the age of 7 months when he was admitted to the Pediatric University Clinic in Odense Studies of the amino acid pattern of blood urine and cerebrospinal fluid by means of ion exchange chromatography showed the aminoaciduria to be of a fairly selective type

METHODS

Specimens of blood urine and cerebrospinal fluid were sent from Odense to the Perinatal Research Laboratory in Uppsala and deproteinized within 36 hours of the time of sampling Deproteinization was accomplished by adding 100 mg of solid sulphosalicylic acid to each 2 ml sample of serum or cerebrospinal fluid Following thorough mixing the samples were centrifuged the supernatant being stored at -20 C until processed. Urine specimens were stored at the same temperature

Amino acid concentrations were determined by means of either of two automatic amino acid analyzers one built at the Laboratory to the specifications of Spackman et al (16) and one commercial (Biocal BC 200) Both apparatuses permitted analysis to be carried out on 0.2 ml of deproteinized serum and on 1.0 ml of deproteinized cerebro-spinal fluid

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Case Report

A C male infant born on May 26th 1967 He was the third child of healthy non related parents The parents first child was a stillborn boy the second a healthy now 7 year-old girl An investigation of the family disclosed five additional cases of Lowe's syndrome the subject of another report (12)

The patient was born at term after a pregnancy uneventful except for the occurrence of mild transitory vaginal bleeding in the fourth month Delivery was uncomplicated birth weight 3 600 g and length (CH) 56 cm The testes were not descended Shortly following birth ocular clouding as well as marked muscular hypotonia were noted and at the age of 11 days an ophthalmological examination confirmed the presence of bilateral cataract

At the age of 25 days the boy was admitted to the Pediatric University Clinic in Odense where he is still (March 1970) an in patient. His psycho-motor development has been severely retarded and since the age of 3 months his weight has been below the 2.5 percentile The muscular hypotonia noted at birth is associated with hyperflexibility of the joints and with absence of tendon reflexes He is now almost 3 years old and weighs 9 kg The length is 78 cm head circumference 52 cm chest circumference 46 cm He is blind and profoundly oligophrenic (Fig 1)

In addition to the cataract severe bilateral *glau*



Fig 1 The patient one year old

coma with enlargement and progressive clouding of the corneas and with very high pressures developed during the first year of life and several unsuccessful attempts at medical or surgical alleviation of the ocular hypertension including goniotomies and evacuation of the right anterior camera were made

Intravenous urographies disclosed a left distal *ureteral stricture* with marked dilatation of the left urinary tract At the age of 7 months surgical procedures including plastic operations on the left ostium and on the vesical collum were performed Urinary tract infections have been treated early and the serum concentrations of creatinine and urea remain normal

The presence of *renal rickets* was demonstrated at the age of 7 months when treatment with vitamin D was initiated *The occurence of rachitic bone changes* was preceded by the development of a *metabolic acidosis* A blood acid base study at the age of 4 weeks was within normal limits although the blood titratible base whole blood base excess (BE) determined according to Astrup et al (2) was on the negative side −3 7 mEq/l *Three months* later the patient was definitely acidotic the BE being −11 5 mEq/l (standard bicarbonate 15 3 mEq/l) From the age of 10 months the patient was given a citrate buffer (approx 1 000 mEq of potential base per liter) administered orally in doses of 6 to 8 mEq per kg body weight per day A survey of the large number of acid–base studies performed throughout the course reveals a striking lack of effect of this treatment Spontaneous fluctuations of the blood BE level (limits zero and −11 mEq/l) occurred apparently unrelated to the actual level of base administration. At the age of 27 months when the patient was receiving 50 mEq of citrate a day and had a blood BE of approximately −8 mEq/l the rate of renal net acid excretion (7) was +3 6 +0 6 and −5 0 mEq *per 24 hours on three consecutive days When eval*uated in terms of the actual level of blood BE using the data of Kildeberg (8) and an estimated *body surface area of 0 41 m² these values are patho*logically low On other occasions even more negative values (approx −20 mEq per 24 hours) were obtained *Urine pH* values as low as 5 were found following temporary withdrawal of citrate therapy and the rate of renal ammonia production was invariably higher than that of the excretion of titratable acid

Thus the presence of renal acidosis is well established Furthermore the persistently high serum chloride levels (105–120 mmoles/l) the normal serum concentrations of creatinine and of urea as well as the development of rickets serve to characterize the disturbance as a renal *tubular* acidosis In our case neither deficient renal ammonia excretion nor failure to produce a normal plasma/urine pH gradient can be reasonably cited as causes of the metabolic acidosis Rather the failure of base administration in doses equivalent to more than five times the expected rate of endogenous production of acid about 1 mEq per kg per day (8) to correct the acidosis and renal losses of base in the presence of significant acidosis suggest a low renal bicarbonate threshold to be instrumental in the maintenance of the acidotic state

Additional routine studies

Routine studies included normal electrocardiograms and normal electroencephalograms X ray studies of the skeleton including the skull revealed nothing abnormal except for retarded bone age (17 months at the chronological age of 21 months) and rachitic changes subsiding during the second year of life A chromosomal analysis (Dr J Mohr and Dr J Schultz Larsen Copenhagen) showed a normal pattern of chromosomes

The blood hemoglobin concentration varied between 10 0 and 13 1 g per 100 ml the serum creatinine concentration between 0 5 and 0 6 mg per 100 ml A study of the proteins in serum at the age of 11 months gave the following results total concentration 9 4 g per 100 ml (albumin 48 α_1 globulin 3 α_2 globulin 20% β globulin 12 γ globulin 17) Immunoelectrophoresis of the proteins in serum showed increased concentrations of IgA IgG and IgM (Dr J Clausen Copenhagen) The serum concentration of β microglobulin was found to be 2 5 μg per ml (Dr P E Evrin Uppsala)

During the first 3 months of life intermittent

[illegible] was present. Proteinuria has been a constant finding, concentrations varying between 0.9 and 2.5 g/l. An electrophoretic study of the urinary protein at the age of 11 months gave the following results: total concentration 2.2 g/l (albumin 22%, α_1 globulin 10%, α_2-globulin 31%, β globulin 16%, γ-globulin 21%). At the age of 21 months the rate of urinary loss of β_2-microglobulin was 57 mg per 24 hours.

Amino acids

The results of quantitative amino acid analyses on specimens of serum or urine obtained on two different occasions are presented in Tables 1, 2, and 3. The data of Table 1 show that the pattern of amino acids in serum was normal except for a slight increase in the concentration of glutamic acid, possibly due to conversion of glutamine during storage.

However, a marked increase in the excretion of several amino acids such as hydroxyproline, proline, citrulline, alanine and lysine was found in both samples of urine (Table 2). Also the rate of excretion of tyrosine was abnormally high at the age of 21 months when the second urine specimen was obtained, whereas a normal amount of this amino acid was found in the first specimen collected at the age of 13 months. On neither of these occasions could a generalized aminoaciduria be demonstrated since the amino acids not mentioned occurred in normal amounts (Table 2).

Table 1. *Amino acid pattern in the serum*

The values are given in mg per 100 ml

Amino acid	Specimen obtained at the age of 13 months	21 months	Normal range (ref. 15)
Taurine	0.44	1.26	0.71–1.44
Aspartic acid	trace	0.78	0.05–0.27
Threonine	1.71	1.33	0.50–1.13
Serine	2.10	1.79	0.83–1.18
Glutamine/Asparagine	10.4	9.19	8.3–68.0
Proline	2.93	1.53	0.78–1.70
Glutamic acid	[illegible]	2.94	0.34–3.68
Citrulline	trace	0.57	0.21–0.92
Glycine	3.45	3.21	0.88–1.67
Alanine	4.90	3.80	1.2–2.72
Valine	2.48	2.65	1.50–3.31
Cystine	trace	trace	0.34–0.97
Methionine	0.2[illegible]	0.30	0.16–0.24
Isoleucine	0.73	0.77	0.37–1.1
Leucine	1.58	1.57	0.73–[illegible].32
Tyrosine	0.80	0.89	0.59–1.28
Phenylalanine	2.30	1.76	0.43–1.09
Ornithine	1.27	1.73	0.36–1.14
Lysine	1.59	2.39	1.04–[illegible].20
Histidine	0.83	1.27	0.77–1.32
Arginine	1.92	1.36	0.40–1.90

Table 2. *Amino acids in the urine*

The values are given in mg per 24 hours

Amino acid	Specimen obtained at the age of 13 months	21 months	Normal range (ref. 17)
Taurine	trace	7	96–294
Hydroxyproline	59	23	0–trace
Aspartic acid	13	2	10
Threonine	3	146	15–53
Serine	13	Not determined	27–73
Glutamic acid	555	45	8–40
Proline	367	219	10
Citrulline	40	44	—
Glycine	101	186	68–199
Alanine	307	193	21–71
Cystine	29	38	10–21
Valine	trace	27	4–6
Methionine	trace	12	10
Isoleucine	8	trace	14–28
Leucine	4	7	9–26
Tyrosine	trace	118	15–49
Phenylalanine	trace	24	9–31
β-aminoisobutyric acid	20	6	0–28
Ornithine	38	1	—
Lysine	334	310	7–48
Histidine	305	206	113–320
Arginine	trace	21	—

Table 3 shows calculated renal clearances of the various amino acids. Corresponding normal values for children at different ages reported by others (3, 11, 15) are presented for comparison. The data demonstrate the absence of a generalized aminoaciduria in our case. It has not been possible to derive exact renal clearances of hydroxyproline, but they are very high for both specimens.

Interestingly, at 13 months of age only the renal clearances of citrulline, glycine and cystine were above those reported for healthy premature infants, although when compared with clearance values valid for infants of more than 4 months of age the clearances of proline and lysine are also high. Correspondingly, at the age of 21 months the renal clearances of hy-

Table 3 *Renal clearances of amino acids*

Values given as ml per min

Amino acid	Specimens obtained at the age of 13 months	21 months	Premature infants (Ref 11)[a]	Infants 16 days–4 months (Ref 3)[b]	Children 2–13 years (Ref 3)[b]	Children 3–10 years (Ref 15)
Taurine	Not determined	0 4	0 6–1 3	—	—	9 9–26 2
Hydroxyproline	Not determined	High	16	—	—	Low
Aspartic acid		0 2	4 2 (1)	—	3 6±0 9	Tr–8 8
Threonine	0 03	7 6	3 0–8 2	2 0±1 4	1 0±0 2[c]	0 5–2 5
Serine	Not determined	Not determined	4 6–9 3	4 1±1 9	2 4±0 5	1 2 3 4
Glutamic acid	2 9	1 1	—	—	—	0 1–2 4
Proline	1 8	9 7	2 1–13	1 0±0 7	0 4±0 2	0–0 3
Citrulline	High	5 3	0 6–2 5	—	—	0 6 (1)
Glycine	High	4 0	12 –26	7 4±3 2	4 2±1 4	1 2–8 6
Alanine	0 9	3 5	1 5–3 7	1 7±0 8	0 8±0 4	0 2–1 3
Cystine	High	High	3 6–8 8 (2)	1 1±0 3	0 8±0 2	1 0–1 4
Valine	Low	1 0	0 2–0 6	0 3±0 1	0 2±0 1	0 1–0 3
Methionine	Low	0 3	2 3–5 8	0 8±0 6	0 8±0 3	1 0–3 4
Isoleucine	0 2	Low	0 4–1 0	0 3±0 2	0 3±0 1	0 2 1 0
Leucine	0 04	0 3	0 4–1 3	0 8±0 5	0 5±0 2	0 2–0 9
Tyrosine	Low	9 2	0 6–2 6	1 8±0 4	2 0±0 8	0 8–3 3
Phenylalanine	Low	0 9	1 2–2 2	1 7±0 9	1 5±0 3	0 3–2 3
Ornithine	0 4	0 04	0 9–1 0 (3)	0 4±0 1	0 4±0 1	0 2–0 8
Lysine	3 1	9 0	1 6–5 2	1 3±0 4	1 2±0 4	0 3–2 4
Histidine	7 0	11 2	7 –19	8 5±4 5	9 5±2 6	1 9–21 8
Arginine	Low	1 1	—	0 4±0 1	0 3±0 1	0 2 1 2

[a] Values given represent range

[b] Values given represent mean ± S D

[c] Threonine + Asparagine

Table 4 *Amino acids in the cerebrospinal fluid*

The values are given in μmoles per 100 ml

Amino acids	Specimen obtained at age 13 months	Normals (Ref 4)
Taurine	0 52	0 22–0 94
Hydroxyproline	—	—
Aspartic acid	Not separated	0 03–0 22
Threonine	Not separated	0 77–4 91
Asparagine/Glutamine	63 2	19 4–86 5
Serine	7 80	1 65–12 1
Glutamic acid	6 6	0 24–2 5
Proline	—	0 –0 58
Citrulline	Trace	0 11–0 45
Glycine	1 0	0 40–1 07
Alanine	2 9	1 17–4 46
β aminoisobutyric acid	—	0 14–0 91
Cystine	Trace	Trace
Valine	2 2	0 74–2 82
Methionine	0 48	0 05–0 75
Isoleucine	0 65	0 30–0 86
Leucine	1 54	0 62–2 22
Tyrosine	1 20	0 30–2 43
Phenylalanine	1 40	0 16–2 74
Lysine	2 87	0 61–3 34
Arginine	2 62	0 80–2 91

droxy-proline citrulline cystine valine, tyrosine and lysine are above those of premature infants but compared with normal infants older than 4 months elevated values are found for several other amino acids including threonine serine proline and alanine Clearances of all other amino acids investigated were within normal limits for both samples

Table 4 shows the amino acid concentrations in a sample of cerebro spinal fluid obtained at the age of 13 months All amino acids studied appeared in normal concentrations, and the serum/CSF molar ratios were similar to those reported for normal adults (5)

DISCUSSION

It has often been stated that the oculo cerebro-renal syndrome is associated with generalized aminoaciduria (1) However up to the present time few complete quantitative studies of urinary amino acids in this syndrome by reliable

methods such as ion exchange chromatography have been published Furthermore even the published figures do not always confirm the presence of unselective renal losses of amino acids

In our patient the aminoaciduria appears to be of a highly selective type with dispropor tionately high clearances for several amino acids including proline lysine hydroxy proline and citrulline Furthermore the data suggest a progression of the aminoaciduria with time although an incidental variation in tubular dysfunction might account for the observed difference between the two samples The data also confirm the renal origin of the aminoacid uria since serum amino acid concentrations were largely normal The figures of Table 3 bear out a conspicuous similarity between the aminoaciduria observed in our patient and the physiologic aminoaciduria of premature in fants suggesting that tubular functional im maturity rather than specific toxic agents may be responsible for the aminoaciduria of pa tients with the Lowe syndrome

In their review Abassi et el (1) reported quantitative data on amino acid concentrations in urine obtained from 7 patients with this syndrome and claimed the presence of genera lized aminoaciduria Their data show that the concentrations must be given in micromoles per 24 hours and not in millimoles per 24 hours as stated in the text since otherwise their normal controls would have had gross aminoaciduria Furthermore in some of their cases the aminoaciduria appears to have been rather specific and selective and similar to that observed in our patient Using paper chro matography Jagenburg (6) studied a type of fairly selective aminoaciduria in the Lowe syn drome similar to the aminoaciduria of classical cystinuria with prominent urinary losses of ly sine ornithine arginine and cystine

According to our present knowledge at least five group-specific enzyme systems are involved in the tubular reabsorptions of filtered amino acids (11) In our cases tubular tansport of the aminoacids proline and hydroxy proline as well as that of lysine and citrulline seems to be impaired whereas reasorption of the neutral amino acids is largely normal How ever in Jagenburg's case (6) reabsorption of the basic amino acids appeared to be specifically blocked Thus the tubular lesion responsible for aminoaciduria in the Lowe syndrome may vary at least initially with respect to the par ticular enzyme systems involved In our pa tient, the magnitude of pathologic β_2-micro-globulinuria was similar to that previously re ported in this syndrome as well as in other types of tubular dysfunction (13)

The bearing if any of the aminoaciduria on the co-existing tubular acidosis in the oculo-cerebro-renal syndrome remains unknown In one case described by McCance et al (10) tubular ammonia production seemed to be de ficient but confirmatory reports have not ap-peared In our patient a low renal bicarbonate threshold appears to be responsible for the acidosis this would correspond well to a prox imal level of tubular dysfunction

Since the Lowe syndrome involves the cen tral nervous system and since the distribution of aminoacids between plasma and cerebro-spinal fluid is believed to be dependent on active transport mechanisms concentrations of amino acids in a sample of cerebrospinal fluid were determined As shown in Table 4 no abnormalities were found On the other hand in some hydranencephalic patients studied by us distinctly abnormal cerebrospinal concen trations of various amino acids have been ob-served (18)

SUMMARY

Ion exchange chromatographic studies of the distribution of a number of amino acids in the body fluids of a two-year-old boy with the oculo-cerebro-renal syndrome are reported Amino acid concentrations in serum and in cerebrospinal fluid were normal whereas stud ies of the urine disclosed a highly selective (renal) aminoaciduria rather similar to that occurring in healthy premature infants

Complementary observations on the acid-base metabolism served to establish the presence of renal tubular acidosis, probably due to a low tubular bicarbonate threshold

The implications of these findings are briefly discussed

REFERENCES

1 Abitu V Lowe C U & Calcagno P L Oculo cerebro renal syndrome *Amer J Dis Child 115* 145 1968

2 Astrup P Jorgensen K Siggaard Andersen O & Engel K The acid–base metabolism A new approach *Lancet I* 1035 1960

3 Brodehl J & Gelissen K Endogenous renal transport of free amino acids in infancy and childhood *Pediatrics 42* 395 1968

4 Dickinson J C & Hamilton P B The free amino acids of human spinal fluid determined by ion exchange chromatography *J Neurochem 13* 1179 1966

5 Humoller F L Mahler D J & Parker M M Distribution of amino acids between plasma and spinal fluid *Int J Neuropsych 2* 293 1966

6 Jagenburg O R The urinary excretion of free amino acids and other amino compounds by the human *Scand J Clin Lab Invest 11* Suppl 43 150 1959

7 Jorgensen K Titrimetric determination of the net excretion of acid base in urine *Scand J Clin Lab Invest 9* 287 1957

8 Kildeberg P *Clinical acid base physiology Studies in neonates infants and young children* Munksgaard Copenhagen 1968

9 Lowe C U Terrey M & McLachlan E A Organic aciduria Decreased renal ammonia production hydrophthalmus and mental retardation A clinical entity *Amer J Dis Child 84* 164 1952

10 McCance R A Matheson W J Gresham G A & Elkinton, J R The cerebro-ocular renal dystrophies A new variant *Arch Dis Child 35* 240 1960

11 O'Brien D & Butterfield L J Further studies on renal tubular conservation of free amino acids in early infancy *Arch Dis Child 38* 437 1963

12 Pallisgaard G & Goldschmidt E The oculo cerebro renal syndrome of Lowe in four generations of one family *Acta Paediat Scand* 1971

13 Peterson P A Evrin P E & Berggård I Differentiation of glomerular tubular and normal proteinuria Determination of urinary excretion of β₂-microglobulin albumin and total protein *J Clin Invest 48* 1189 1969

14 Scriver C R Amino acid transport in the mammalian kidney *In* W L Nyhan (ed) *Amino acid metabolism and genetic variation* McGraw Hill New York 1967 p 327

15 Scriver C R & Davies E Endogenous renal clearance rates of free amino acids in prepubertal children *Pediatrics 36* 592 1965

16 Spackman D H Stein W H & Moore S Automatic recording apparatus for use in the chromatography of amino acids *Anal Chem 30* 1191 1958

17 Stein W H A chromatographic investigation of the amino acid constituents of normal urine *J Biol Chem 201* 45 1953

18 Ulwån O Hambraeus L & Andersson G The aminogram of cerebrospinal fluid in a case of hydranencephaly before and after shunt operation In preparation

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FATTY ACID COMPOSITION OF PLASMA AND RED CELL PHOSPHOGLYCERIDES IN FULL TERM INFANTS AND THEIR MOTHERS[1]

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There is evidence that malnutrition during early life can lead to persisting intellectual and emotional disturbances (2 10) Almost all of the relevant studies have been concerned with protein-calorie deficiencies during the first period of life nutritional status during the gestational period and the influence of other nutrients having received relatively little attention Although the brain is built largely of lipids with a high concentration of polyunsaturated fatty acids which are essential for mammals very little is known about the requirements of essential fatty acids for normal development of the foetal brain

Knowledge about the supply of the human foetus with nutrients essential for normal development is incomplete Between the maternal and foetal side of the placenta there are puzzling concentration gradients of many compounds e g higher level of blood glucose and free amino acids at the foetal side (23) On the other hand it has long been known that the concentrations of plasma lipids are much lower in the newborn than in the mother (26)

No systematic studies of the serum fatty acid composition of well nourished mothers and their infants were known at the beginning of the present study of the blood phosphoglycerides This lipid class was chosen instead of total lipids in order to reduce the influence of rapid and transient changes in environmental conditions which were assumed to be reflected more instantly in the fatty acid composition of free fatty acids and triglycerides than in that of the phosphoglycerides

MATERIAL AND METHODS

Clinical material

The series consisted of 20 apparently healthy pregnant women Ten of them were selected at random from the Prenatal Care Unit of the Obstetric Service I Sahlgren s Hospital Goteborg The women were instructed to keep a dietary record for each week which was then checked at an interview by a trained dietitian Their diets did not differ significantly from that of the general Swedish population The remaining 10 women were selected at parturition All of the women had uncomplicated pregnancies and deliveries Seven of the women were primiparae 9 were diparae and 4 were triparae The mothers ages and the gestational ages are given in Table 1 The newborn infants showed normal respiratory and circulatory adaptation immediately after birth and uneventful neonatal courses They were discharged from the hospital 4 to 6 days after birth All the newborns were of fullterm normal appearance except one with a gestational age of 42 weeks who looked somewhat dysmature Birthweights lengths at birth and placental weights are given in Table 1

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Adsorbents Silicic acid (Mallinckrodt A. R.) 300

[1] A preliminary report of the present data was given at the Semper Symposium of Nutrition Wenner Gren Centre Stockholm 1969

Complementary observations on the acid base metabolism served to establish the presence of renal tubular acidosis, probably due to a low tubular bicarbonate threshold

The implications of these findings are briefly discussed

REFERENCES

1 Abassi V Lowe C U & Calcagno P L Oculo cerebro renal syndrome *Amer J Dis Child 115* 145 1968
2 Astrup P Jørgensen K Siggaard Andersen O & Engel K The acid–base metabolism A new approach *Lancet I* 1035 1960
3 Brodehl J & Gellissen K Endogenous renal transport of free amino acids in infancy and childhood *Pediatrics 42* 395 1968
4 Dickinson J C & Hamilton P B The free amino acids of human spinal fluid determined by ion exchange chromatography *J Neurochem 13* 1179 1966
5 Humoller F L Mahler D J & Parker M M Distribution of amino acids between plasma and spinal fluid *Int J Neuropsych 2* 293 1966
6 Jagenburg O R The urinary excretion of free amino acids and other amino compounds by the human *Scand J Clin Lab Invest 11* Suppl 43 150 1959
7 Jørgensen K Titrimetric determination of the net excretion of acid base in urine *Scand J Clin Lab Invest 9* 287 1957
8 Kildeberg P *Clinical acid base physiology Studies in neonates infants and young children* Munksgaard Copenhagen 1968
9 Lowe C U Terrey M & McLachlan E A Organic aciduria Decreased renal ammonia production hydrophthalmus and mental retardation A clinical entity *Amer J Dis Child 84* 164 1952
10 McCance R A Matheson W J Gresham G A & Elkinton J R The cerebro-ocular renal dystrophies A new variant *Arch Dis Child 35* 240 1960
11 O Brien D & Butterfild L J Further studies on renal tubular conservation of free amino acids in early infancy *Arch Dis Child 38* 437 1963
12 Pallisgaard G & Goldschmidt E The oculo-cerebro renal syndrome of Lowe in four generations of one family *Acta Paediat Scand* 1971
13 Peterson P A Evrin P E & Berggård I Differentiation of glomerular tubular and normal proteinuria Determination of urinary excretion of β_2-microglobulin albumin and total protein *J Clin Invest 48* 1189 1969
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Table 1 *Age and period of pregnancy of the mothers birth weight and length of the newborns and placental weights*

Number = 20	Mean	Range
Mothers		
Age	27 years	17–36 years
Gestational age	40 4 weeks	40–42 weeks
Newborns		
Birthweight	3 314 g	2 790–3 990 g
Length at birth	49 3 cm	47–53 cm
Placental weight	537 g	400–780 g

mesh was passed through a 325 sieve freed from impurities and fines by suspension in methanol and activated at 120 C (52) A 0 25 mm layer of Silica Gel (E Merck A G Darmstadt) was spread on a glass plate 20 × 20 cm and activated overnight at 120 C

Quantitative determination of lipids

Lipid phosphorus was assayed by a Bartlett procedure (3) cholesterol by the method of Crawford (11) and triglycerides by the method of Carlsson (8) Phospholipid concentration was calculated by multiplying the value for lipid phosphorus by 25 (51)

Blood sampling

Immediately after the cord had been clamped blood from the placental stump of the umbilical cord was allowed to run freely into a heparinized test tube care being taken to avoid admixture of maternal blood The sample was collected while the placenta was still in the uterus A maternal venous blood sample was obtained from an antecubital vein within 5 min of delivery

The plasma was separated from the blood cells within 4 hours by centrifugation The plasma was removed by suction and the red cells were washed twice with 2 to 3 volumes of physiological saline After the last centrifugation at 2 500 × g for 10 min 0 5 ml of the packed and washed red cell sediment was transferred to a new test tube The red cells were then hemolyzed by addition of 10 ml of 0 005 M phosphate buffer pH 6 8 and the tube centrifuged at 3 000 × g for 60 min The red and clear supernatant was removed from the slightly pink sediment of the red cell stroma

Preparation of lipid extract

Six ml of C M 1 1 was added to the test tube with red cell stroma which was then shaken vigorously In the same way 6 ml of C M 1 1 was added to 0 5 ml of plasma The tube was centrifuged at 2 000 × g for 10 min and the extract was transferred to a tube with ground glass stopper The sediment was re extracted with 2 ml of C M 1 1 The two extracts were pooled and 4 ml of chloroform and 2 ml of acid physiological saline (0 5 g sodium chloride and 0 5 ml 1 M sulfuric acid dissolved into 500 ml of distilled water) was added after which the tube was vigorously shaken for 1 min and centrifuged (2 000 × g) for 10 min The upper phase was removed and the lower phase was washed with rinsing solution (15 ml of chloroform 260 ml of methanol and 250 ml of 0 1% sodium chloride) and then transferred to a 10 ml cylinder The volume was made up to exactly 10 ml with C M 2 1 Duplicate aliquots of 0 2 ml were used for determination of the lipid phosphorus

Separation of lipids by column chromatography with silicic acid

The lipid extract (9 6 ml) was transferred to a round bottomed flask and evaporated to dryness under reduced pressure in a rotating evaporator at a temperature not exceeding 40 C The lipids were immediately redissolved in 5 ml of chloroform The chloroform extract was added to a column containing 1 g of silicic acid The column was prepared by suspending the silicic acid into chloroform and stirring it into a glass column with an inner diameter of 6 mm The flask was rinsed twice with 2 ml of chloroform which was added to the column When the lipid extract had entered the column the elution of neutral lipids was completed by adding 15 ml of C M 19 1 for plasma and 15 ml of C M 50 1 for red cell stroma The effluent was collected into a 25 ml cylinder The plasma phospholipids were eluted with 25 ml of methanol The red cell phospholipids were separated into cephalins and choline phospholipids The former were eluted by 25 ml of C M 3 1 and the latter by 25 ml of methanol The separation was checked by thin layer chromatography on a silica gel plate which was developed with C M W 65 25 4 The spots were visualized by spraying with anisaldehyde solution (36) Duplicate aliquots of the neutral lipid eluates were used for quantitative determination of triglycerides and cholesterol and duplicate aliquots of the phospholipid eluates for quantitative determination of phospholipids Aliquots of the phospholipid eluate equivalent to about 10 µg of lipid phosphorus were evaporated to dryness under nitrogen in conical tubes with ground glass stoppers

Separation of lipids by thin layer chromatography on silica gel

Portions of the plasma lipid extract corresponding to 10–20 µg of lipid phosphorus were applied as 2–3 cm broad bands on 20 × 20 cm thin layer plates coated with silica gel The plates were developed with C M W 65 25 4 for the isolation of lecithins and with light petroleum–diethyl ether–acetic acid 90 10 1 for isolation of total plasma phosphoglycerides The spots were visualized by spraying with bromothymol blue reagent (31) The spots with lecithins or total phosphoglycerides respectively were quantitatively scraped into glass tubes and placed into a vacuum desiccator which was immediately evacuated and allowed to stand over night (53)

analysis of phosphoglycerides

preparation of methyl esters of the phosphoglyc and their quantitative assay by GLC were med with the standard technic of the laboratory Two ml of 0 1 M sodium methoxide in dry nol was added and the tubes were gently shaken hour at room temperature For neutralization ml of 1 M acetic acid was added and the methyl s were extracted three times with 2 ml of light oleum b p 45–50 C The light petroleum ex s were pooled in conical tubes with ground glass pers and washed with distilled water The water removed from the bottom by suction To remove water the tubes were frozen to −20 C and the t petroleum decanted off into new tubes

C of methyl esters

extract with the fatty acid methyl esters was porated to about 100 µl on a water bath at 25 C ler a gentle stream of nitrogen and then transfer to a conical microtube (volume 300 µl) with glass pper It was then evaporated further to a final ume of about 10 µl One µl was analysed in a kin Elmer Model 880 apparatus on a 200 cm inless steel column of 33 mm i d packed with diethylene glycol succinate polyester (DEGS) ted on Gaschrom Q 80-100 mesh Helium was d as carrier gas and the column temperature was 0 C The peaks in the chromatograms were identi d and quantitated in the way described previously)

tistical methods

e mean standard deviation of the concentrations main plasma lipid classes and of the fatty acid ncentrations in phosphoglycerides were calculated cause it cannot be postulated that the values for others and newborns are independent the statistical nificance of the differences was assessed from the ferences in fatty acid concentrations in the partic lar pairs of mother–newborn It was postulated that e differences in concentrations were normally distr uted For the correlation analyses it was supposed at the distribution of fatty acid concentrations and ncentrations of cholesterol and phospholipids in others and newborns constituted two-dimensional rmal distributions (48)

RESULTS AND DISCUSSION

Concentrations of main lipid classes in plasma

The mean values and standard deviations found for the plasma lipids are given in Table 2 The total lipids were calculated as the sum of the different lipid classes The cholesterol *figure* was multiplied by 1 44 to include the fatty acids in the cholesterol esters and for *free* fatty acids figures of 20 mg/100 ml and 10 mg/100 ml were added for mothers and newborns respectively

Table 2 *Mean concentration of plasma lipids at birth*

	Mother (mg/100 ml) (n = 20)		Infant (mg/100 ml) (n = 20)	
Constituent	Mean	S D	Mean	S D
Total lipids	886	126	257	59
Triglycerides	179	58	39	20
Cholesterol	257	39	66	18
Phospholipids	317	36	113	24
Cholesterol/ phospholipid ratio	0 81	0 06	0 59	0 07

Mothers The mean cholesterol concentration of 257 mg/100 ml is in good agreement with the mean of earlier investigations of 272 mg/100 ml (4 7 14 21 22 29 30 32–35 44 47 54 57) The range of the means in earlier investigations was wide 208–357 mg/100 ml but in 11 of the 17 studies it was 240 to 275 mg/100 ml The total phospholipid concentration of 317 mg/100 ml found in the present study also agrees well with the mean of earlier investigations of 306 mg/100 ml range 192–406 mg/100 ml (13 14 21 22 30 32 34 42 44 46 58) The phospholipid concentration of 406 mg/100 ml reported by Renkonen (44) from Finland is the highest on record and might be due to some unknown source of error because at this concentration the cholesterol/phospholipid quotient would be very low 0 64 In previous studies a mean cholesterol/phospholipid quotient of 0 89 can be calculated which is slightly higher than the present ratio of 0 81 The present value for triglycerides of 179 mg/100 ml is lower than the mean found in earlier studies viz 249 mg/100 ml range 159–302 mg/100 ml (13 14 32 34 44 47 54) No relation was found in earlier publications between the time of collection of the blood (just b fore or after delivery) and the l vel of triglycerides

Newborns The present values for triglycerides cholesterol and total phospholipids are in good agreement with the mean values ob-

Table 3 *Fatty acid composition of infant blood plasma phosphoglycerides isolated by three different methods*

Fatty acid	Phosphoglycerides (n = 10) Column (A) Mean	S D	TLC (B) Mean	S D	Lecithin (n = 10) TLC (C) Mean	S D	Differences (B) (A) Mean		Differences (C)–(A) Mean	
16 0	29 6[a]	0 49	30 9	0 98	30 9	1 74	+1 2	*[b]	+1 2	[b]
16 1	1 7	0 28	1 6	0 33	1 6	0 44	0 0	–	–0 2	–
18 0	14 7	0 96	15 0	1 20	14 3	0 80	+0 3	–	–0 5	**
18 1	12 7	1 33	12 8	1 34	12 7	1 53	0 1	–	0 0	–
18 2 (n 6)	8 5	1 52	8 7	1 36	8 8	1 16	+0 2	–	+0 3	–
20 3 (n–6)	5 5	0 72	5 4	0 86	5 7	0 71	–0 1	–	+0 1	–
20 4 (n–6)	16 6	1 72	15 4	1 59	15 7	1 82	–1 1	**	–0 7	*
22 6 (n 3)	7 2	1 19	6 8	1 03	7 2	1 88	–0 4		+0 1	–
18-22 (n 6)	32 2	1 21	30 9	1 80	31 5	1 63	–1 2	**	–0 7	–

[a] Values are weight percentages of methyl esters
[b] Stands for significance level – 0 05 level * – 0 01 level ** – 0 001 level

tained in earlier investigations for triglycerides 50 mg/100 ml range 33–87 mg/100 ml (5 6 32 34 44 54) for cholesterol 73 mg/100 ml, range 34–98 mg/100 ml (4–7 12, 19–22, 25 29, 30 32–35 38 40 43–45 49 54 59) and for total phospholipids 119 mg/100 ml, range 61–212 mg/100 ml (5 6 9 12, 19–22, 30 32, 34 38 40 42–45, 54 59) The cholesterol/phospholipid ratio in the aforementioned earlier studies as calculated by us was 0 66 compared with 0 59 in the present study The present figures for cord blood were very close to those in previous Scandinavian studies by Brody & Carlsson (6) and by Renkonen (44) No correlation was found between the plasma cholesterol or phospholipid levels in the mothers and their newborns The triglycerides were not studied for any such correlation it being known that they have a skew distribution (6)

Fatty acid composition of blood phosphoglycerides

Comparison of methods A Isolation of plasma phosphoglycerides There are two principal methods for the isolation of total plasma phospholipids column and thin layer chromato

Table 4 *Fatty acid composition of mother blood plasma phosphoglycerides isolated by three different methods*

Fatty acid	Phosphoglycerides (n = 10) Column (A) Mean	S D	TLC (B) Mean	S D	Lecithin (n = 10) TLC (C) Mean	S D	Differences (C)–(A) Mean	
16 0	30 1	1 48	31 1	1 01	32 1	1 57	+2 1	*[b]
16 1	1 6	0 30	1 5	0 37	1 5	0 42	–0 1	–
18 0	11 4	1 06	11 4	0 97	10 0	0 98	–1 5	*
18 1	14 2	1 47	14 8	1 53	14 4	1 50	0 2	–
18 2 (n–6)	22 2	2 82	22 2	3 20	23 4	3 15	+1 2	*
20 3 (n 6)	3 2	0 54	3 1	0 45	3 2	0 56	0 0	–
20 4 (n–6)	8 9	1 42	8 2	1 29	7 8	1 51	–1 0	*
22 6 (n–3)	5 6	0 82	5 1	0 63	4 9	0 72	–0 7	*
18–22 (n–6)	34 9	2 57	34 0	3 15	35 0	2 56	+0 2	–

[a] and [b] see text in Table 3

graphy. The latter method has been more widely used during the last few years because it is simpler and more sensitive. When mild alkaline transmethylation is used only the phosphoglyceride fatty acids will be transmethylated. Since different phosphoglycerides have their own characteristic fatty acid pattern there is also an increasing tendency to determine the fatty acid composition of individual phosphoglycerides. Lecithin is the predominating phosphoglyceride of plasma and constitutes more than 90 % of the total plasma phosphoglyceride fatty acids (41). We therefore thought it worth while to find out whether the two isolation procedures for total plasma phosphoglycerides and for lecithin gave comparable fatty acid data. Phospholipids from 10 individual samples of maternal plasma and umbilical cord plasma were isolated by three different methods: on silicic acid column according to the procedure described (A), by thin layer chromatography in light petroleum–ethyl ether–acetic acid 90 : 10 : 1 v/v/v and isolation of the total phospholipid fraction (B), and by thin layer chromatography in chloroform–methanol–water 65 : 25 : 4 v/v/v and isolation of the lecithin spot (53). The results are given in Tables 3 and 4. When the total phospholipids were isolated by column and thin layer chromatography some significant differences were found for the fatty acid composition of the infant blood plasma phosphoglyceride fraction. The TLC procedures gave lower concentrations of 20 : 4 (n–6) and 22 : 6 (n–3). In the TLC method (B) the phospholipids remained at the starting point together with non lipid contaminants. It is probable that heavy metals and particularly hemoglobin derivatives caused an auto-oxidation of the two above mentioned highly polyunsaturated fatty acids (24). In a previous study (53) no difference was found in the brain phosphoglyceride fatty acid pattern whether anti oxidants were added to the lipid extracts or not, but it is possible that such an addition would have had some effect in this study because the phospholipids did not migrate from the contaminants. The differences in fatty acid distribution between total phosphoglycerides and lecithins in umbilical cord blood were so small (Table 3) that the methods can be used interchangeably.

In the isolation of total phospholipids in maternal plasma thin layer chromatography (B) gave lower values for 20 : 4 (n–6) and 22 : 6 (n–6) than did column chromatography method (A), just as it did for umbilical cord plasma. Since the percentages of these acids are lower in the maternal plasma the difference may be ignored. The fatty acid pattern of total phosphoglycerides (A) and that of lecithin (C) differed slightly more in the maternal plasma than in the infants' plasma. In lecithin 16 : 0 and 18 : 2 were higher and 20 : 4 (n–6) was lower than in the total phosphoglycerides ($p < 0.001$). However, the standard deviation of the individual fatty acid levels was much lower than in previous studies (44, 58) so that the three methods can beused interchangeably also for the maternal plasma.

B. *Extraction of red cell lipids.* The lipids of red cells were extracted from saline washed intact cells by the "procedure III" method of Ways & Hanahan (55) or from red cell stroma obtained by lysis in weak phosphate solution (see under Methods). Extraction of red cell stroma instead for intact red cells was faster and easier and the volume of extraction solvent could be strongly reduced. The yield of total phospholipids was the same or slightly better from the stroma than from the intact cells. The fatty acid patterns were very similar with the two methods but in most of the samples the concentration of polyenoic acids were somewhat higher in the stroma extracts. This difference was most pronounced for the cephalin fraction.

It was thus evident that the extraction of red cell stroma instead for intact cells had several advantages and reduced the risk for oxidation catalysed by heme derivatives of the most polyunsaturated fatty acids.

Maternal plasma phosphoglycerides and red cell lecithins. The mean and standard deviations of the fatty acid composition of plasma phosphoglycerides are given in Table 5. The highest concentrations were found for 16 : 0

Table 5 *Fatty acid composition of plasma phosphoglycerides and red blood cell lecithin in mother and infant*

	Plasma phosphoglycerides						Red blood cell lecithin					
	Mother (n = 20)		Infant (n = 20)		Difference Infant Mother		Mother (n = 10)		Infant (n = 9)		Difference Infant Mother	
Fatty acid	Mean	S D	Mean	S D	Mean		Mean	S D	Mean	S D	Mean	
16 0	31 3[a]	2 26	30 2	1 37	−1 1	*[b]	36 4	1 86	36 5	2 02	+0 2	–[b]
16 1	1 4	0 40	1 5	0 38	+0 1	–	1 6	0 51	1 6	0 58	−0 1	–
18 0	11 5	0 92	15 2	1 05	+3 7	*	10 2	0 93	11 9	0 79	+1 8	**
18 1	14 3	1 52	12 1	1 31	−2 1	***	19 4	1 29	18 8	1 70	−0 6	–
18 2 (n–6)	22 1	2 54	8 8	1 31	−13 3	***	18 1	2 40	7 8	1 50	−10 2	* *
18 3 (n–3) + 20 1	1 0	0 28	0 4	0 11	−0 6	*	1 2	0 30	0 4	0 14	−0 8	**
20 3 (n–9)	0 2	0 09	0 6	0 39	+0 4	**	Traces		0 3			
20 3 (n–6)	3 1	0 54	5 5	0 75	+2 3	***	2 3	0 50	3 9	0 63	+1 6	*
20 4 (n–6)	8 5	1 34	16 7	1 40	+8 2	***	6 4	0 55	12 4	1 31	+6 0	*
20 5 (n–3)	0 7	0 19	0 4	0 31	−0 3	*	0 5	0 25	0 3	0 19	−0 2	
22 4 (n–6)	0 2	0 09	0 7	0 15	+0 4	**	0 4	0 08	0 8	0 26	+0 4	*
22 5 (n–6)	0 3	0 14	0 6	0 24	+0 2	*	0 3	0 32	0 5	0 28	+0 2	–
22 5 (n–3)	0 6	0 17	0 5	0 19	0 0	–	0 5	0 20	0 4	0 14	−0 1	–
22 6 (n–3)	4 8	1 06	6 8	1 39	+2 0		2 5	0 73	4 2	1 12	+1 8	*
18–22 (n–6)	34 3	2 48	32 4	1 65	−2 0		27 6	2 06	25 4	1 77	−2 0	
18–22 (n–3)	7 1	1 15	8 1	1 46	+1 0	**	4 6	0 90	5 4	1 33	+0 7	

[a] and [b] see text in Table 3

18 2, 18 1 and 18 0 in decreasing order The fatty acid composition of phosphoglycerides of maternal plasma at term has so far been determined only by Renkonen (44) and Zee (58), who studied plasma lecithin Direct comparison of fatty acid data can often be only approximate because the percentage composition of the fatty acids depends on the number of fatty acids recorded The former two investigators analysed a smaller number of fatty acids than we The values found by them are in general agreement with ours, but they found a *lower concentration of linoleic* acid and the other polyunsaturated fatty acids, and a higher concentration of saturated and monoenoic acids than we Since the differences were largest for the most polyunsaturated fatty acids, a certain loss of these fatty acids during isolation in their studies is a more probable explanation than any difference in the dietary supply of polyunsaturated fatty acids

The figures recorded for maternal plasma phosphoglycerides agreed closely with those found in studies in normal adults, mainly males (24 39, 46 59) though Phillips & Dodge (41) found slightly higher figures for 18 2 and 20 4 In a study of adult males (1) in our laboratory with exactly the same method and performed at the same time as the present investigation 18 2 was higher and 20 4 was lower than in the maternal plasma It is not possible to assess the significance of the small differences found in these two studies because the physiological variation of the phosphoglyceride fatty acid patterns with age sex season, diet etc are not known

The maternal red cell lecithins contained roughly the same percentages of 20 4 and 22 6 as did those found in earlier studies of red blood cells in normal adults (16 55 56) The concentration of 20 3 (n–6) was slightly higher in the maternal samples while the concentration of 18 2 was somewhat lower Red blood cell lecithin contained considerably lower concentrations of 18 2 20 4 and 22 6 than did plasma phosphoglycerides and higher concentrations of 18 1 and 16 0 This is in accordance with the results found by Dodge & Phillips (16) for five adult males

In the present study a significant correlation

Table 6 *Fatty acid composition of red blood cell cephalins in mother and infant*

	Mother (n=10)		Infant (n=9)		Difference Infant Mother	
Fatty acid	Mean	S D	Mean	S D	Mean	
16:0	13.9[a]	1.14	15.4	2.58	+1.7	*
16:1	1.0	0.22	1.2	0.36	+0.2	
18:0	19.7	1.20	20.6	0.93	+0.9	
18:1	17.0	0.78	12.3	1.01	−5.0	
18:2 (n-6)	4.2	0.63	1.8	0.36	−2.4	*
18:3 (n-3)+20:1	1.2	0.17	0.5	0.14	−0.7	
20:3 (n-9)	Traces		0.8	0.20		
20:3 (n-6)	1.6	0.22	2.3	0.26	+0.7	
20:4 (n-6)	20.8	0.99	24.3	1.89	+3.5	
20:5 (n-3)	1.0	0.28	0.3	0.12	−0.8	–
22:4 (n-6)	4.1	0.70	6.6	1.00	+2.6	
22:5 (n-6)	0.8	0.31	1.8	0.52	+1.1	
22:5 (n-3)	3.8	0.52	1.4	0.33	−2.6	
22:6 (n-3)	10.7	0.86	10.8	1.10	+0.1	–
18-22 (n-6)	31.6	1.70	36.9	2.34	+5.3	
18-22 (n-3)	16.8	1.49	12.9	1.36	−3.9	

a and b see text in Table 3

(r=0.768) (n=10) at the 0.01 level was found between the concentration of 18:2 in maternal plasma phosphoglycerides and that in red cell lecithin. There was no significant correlation at the 0.05 level (r=0.357) between the concentration of 20:4 in maternal plasma phosphoglycerides and that in red cell lecithin.

Newborns' plasma phosphoglycerides and red cell lecithin. The fatty acid composition of umbilical cord plasma phosphoglycerides (Table 5) corresponds well with that found by Zollner (59) in 7 cord sera although 22:6 (n-3) was lower in their study. Renkonen (44) and Zee (58) found lower figures for 20:4 and 22:6 (the latter acid was not determined by Zee).

The fatty acid composition of umbilical cord red cell lecithin differed from that of plasma in exactly the same way as that of the maternal plasma and red blood cell: 18:2, 20:4 and 22:6 were lower and 16:0 and 18:1 were higher in red cell lecithin than in plasma phosphoglycerides. The fatty acid pattern found in the present study agreed well with that reported by Crowley, Ways & Jones (12).

There was a significant correlation between concentration of linoleic acid in plasma phosphoglycerides and that in red cell lecithin at the 0.01 level (r=0.803) (n=10) and for arachidonic acid at the 0.05 level (r=0.722, n=10).

Mothers and newborns red cell cephalins. The cephalins differed from lecithin by a high level of arachidonic acid and a very low level of linoleic acid. The ratio between the two fatty acids was 5:1 for cephalin compared to 1:3 for lecithin of the maternal red cells and 12:1 and 1.5:1 respectively for the newborns cells. The sum of the fatty acids of the linoleic acid series 18–22 (n-6) was slightly higher in the maternal red cell cephalins than lecithin but this difference was still higher in the same lipid fractions of the newborns. The sum of the fatty acids of the linoleic acid series was significantly higher at the 0.001 level in the infants' red cell cephalins than in the mothers.

The level of the most polyunsaturated fatty acids of the linolenic acid series was manifold larger in the red cell cephalins than in lecithin of the mothers while the difference was somewhat less in the newborns. The concentration of 22:6 (n-3) was the same in the mothers and infants' red cell cephalins but 22:5 (n-3) was significantly larger in mothers' red cell cephalins. The concentration of all polyunsaturated fatty acids was slightly higher in the

newborns than in the mothers red cell cephalins, 50% and 48% respectively

There are few reports on the fatty acid composition of red cell cephalins in the literature (16 17 55 56) The present figures for the fatty acid composition of mothers red cell cephalins show a very good agreement with the figures of Dodge & Phillips (16) for ethanolamine and serine phosphoglycerides of male adult red cells In our study 16 0 was larger 20 4 (*n*–6) smaller and 22 6 (*n*–3) larger than in their study but the differences were small The fatty acid composition of the newborns red cell cephalins in this study is very similar to that found by Crowley et al (12) for newborns ethanolamine plus serine phosphoglycerides

Mother–infant relation The sum of all fatty acids of the linoleic acid series in plasma phosphoglycerides and red cell lecithin in the infants blood was only slightly lower than in the mothers blood, and the differences were significant only at the 0 01 level In the cephalins this sum was about 25% higher in the newborns than in the mothers, the difference significant at the 0 001 level The concentration of linoleic acid 18 2 (*n*–6) was much lower in both plasma and red blood cells of newborns blood than of maternal blood (Tables 5–6) The lower 18 2 concentration of the cord blood was associated with much higher concentrations of the more unsaturated fatty acids of the linoleic acid series mainly 20 4 (*n*–6) and 20 3 (*n*–6) These findings are in complete agreement with previous reports (44 50 58) A correlation was found between the concentrations of linoleic acid in maternal and cord plasma phosphoglycerides significant at the 0 05 level ($r = 0\ 478$ $n = 20$)

The concentration of fatty acids of the linolenic acid 18 3 (*n*–3) series was higher in cord blood plasma phosphoglycerides and red cell lecithin than in the mothers blood By contrast in the cephalins the sum of the fatty acids of the linolenic acid series was significantly higher in the mothers red cells than in the infants (Table 6) The pattern of the fatty acids of this series showed the same tendency as the linoleic acid series The level of the parent fatty acid 18 3 (*n*–3) was lower in the infants than in the mothers plasma phosphoglycerides and red cell lecithin, but the more polyunsaturated acids were increased The figures for 18 3 (*n*–3) are however uncertain because 18 3 (*n*–3) was not separated from 20 1 with the polar column used for GLC separation of fatty acids

The present study demonstrated very small individual variations in the blood phosphoglyceride fatty acid pattern of mothers in labour and their infants The small variations support the view that environmental factors play at most a minor role compared to a homeostatic mechanism for the regulation of the fatty acid pattern of blood phosphoglycerides The influence of dietary factors on plasma lipids and fatty acids of mothers and their infants was studied by Hansen et al (25) They found no correlation between mother s intake of linoleic acid, varying from 1 5 to 8 6% of calories, and the serum dienoic and tetraenoic acid levels of her infant at birth Renkonen (44) found substantially lower concentrations of the fatty acids of the linoleic acid series in plasma phosphoglycerides of Finnish mothers and their infants He ascribed the low levels of fatty acids of the linoleic acid series to the large proportion of milk fats poor in linoleic acid in the Finnish diet A low recovery of all the polyunsaturated fatty acids better explains his results

In essential fatty acid deficiency 20 3 (*n*–9) is increased (18 28 37) The concentration of this acid was very low in our normal adults but the level was significantly higher in the newborns blood and a slight relative lack of essential fatty acids of the newborns cannot be excluded The most polyunsaturated fatty acids of the linoleic and linolenic acid series were however much higher in the infants than in the mothers blood and the increase of 20 3 (*n*–9) of the oleic acid series might only reflect a generalized increased desaturation and chain elongation of the fatty acids also in this series

The inversed 18 2/20 4 ratio of the lecithin and cephalins in newborns blood com

pared with that in maternal blood might be explained by assumption of a facilitated transport of 20 : 4 prior to that of 18 : 2 from the mother to the foetus or by an increased conversion rate of 18 : 2 to 20 : 4 in the foeto-placental organism. A selective transport of linoleic acid across the placenta has been reported in the guinea pig (27) in which animal in contrast to that seen in humans the percentage of linoleic acid in plasma free fatty acids was approximately 50% greater in the foetus than in the mother. On the other hand an increased activity in the foeto-placental organism of the desaturating and chain elongating enzyme systems which convert the parent fatty acids linoleic and linolenic acids to the more unsaturated C_{20}- and C_{22}-acids might also explain the changed fatty acid pattern.

The physiological significance of the increased unsaturation of the fatty acids in the newborns blood is still unknown. But a consequence is an increased permeability of the red cell membrane (15) which could facilitate the transport of many compounds across the membrane.

SUMMARY

Methods have been adapted and their reproducibility tested for the determination of blood lipids and the fatty acid composition of the phosphoglycerides. The main lipid classes in 20 women from the south western part of Sweden at term pregnancy and of umbilical cord plasma of their newborn infants were studied. Determinations were made of the fatty acid pattern of plasma phosphoglycerides and of cephalins and lecithin of red cells.

The concentration of the major lipid classes in maternal and umbilical cord plasma agreed well with previously reported figures from other countries. The maternal plasma fatty acid pattern of phosphoglycerides was similar to that of adult males. The mothers and their infants had identical concentrations of the total sum of all polyunsaturated fatty acids in plasma phosphoglycerides, in red cell lecithin and in red cell cephalins. The infants pattern differed from the mothers by much lower concentrations of the parent fatty acids linoleic and linolenic acids and correspondingly increased concentrations of the more polyunsaturated fatty acids of the two series. The fatty acid composition in the red cell lecithin of mother and infant reflected that in the corresponding plasma. The cephalins contained more polyunsaturated fatty acids than the lecithin in both mothers and infants red cells.

The study produced no biochemical evidence of an essential fatty acid deficiency in these newborns. The identical concentration of total polyunsaturated fatty acids in blood phosphoglycerides of mothers and infants in combination with the pronounced change in the ratio between the parent fatty acids and the highly polyunsaturated derivates suggest a homeostatic regulation of the fatty acid composition of the blood phosphoglycerides.

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REFERENCES

1 Alling C, Dencker S J, Svennerholm L & Tichy J. Serum fatty acid pattern in chronic alcoholics after acute abuse. *Acta Med Scand* 185: 99, 1969.
2 Barnes R H, Robertson Cunnold S, Zimmermann R, Simmons H, MacLeod R B & Krook L. Influence of nutritional deprivations in early life on learning behavior of rats as measured by performance in a water maze. *J Nutr* 89: 399, 1966.
3 Bartlett G R. Phospholipid assay in column chromatography. *J Biol Chem* 234: 466, 1959.
4 Bersohn I & Wayburne S. Serum cholesterol concentration in newborn afrikan and european

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The inversed 18 2/20 4 ratio of the lecithin and cephalins in newborns blood com

composition of sphingomyelin and lecithin in normal human serum *Proc Soc Exp Biol Med* 119 367 1965

40 Pantelakis S N Cameron A H Davidson S Dunn P M Fosbrooke A S Lloyd J K Malins J M & Wolff O H The diabetic pregnancy A study of serum lipids in maternal and umbilical cord blood and of the uterine and placental vasculature *Arch Dis Child* 39 334 1964

41 Phillips G B & Dodge J T Composition of phospholipids and of phospholipid fatty acids of human plasma *J Lipid Res* 8 676 1967

42 Poli L & Berti G Metodologia nuova per lo studio dei lipidi ematici Valori medi nella madre e nel feto *Pediatria internaz* (Roma) 5 267 1955

43 Rafstedt S Studies on serum lipids and lipoproteins in infancy and childhood *Acta Paediat Scand* 44 Suppl 102 1955

44 Renkonen O V Serum lipids of labouring mothers and newborn babies *Ann Med Exp Biol Fenn* 44 Suppl 10 1966

45 Russ E M Eder H A & Barr D P Protein lipid relationships in human plasma III In pregnancy and the newborn *J Clin Invest* 33 1662 1954

46 Schrade W Bohle E Biegler R Teicke R & Ullrich B Gaschromatographische Untersuchungen der Serumfettsäuren des Menschen III Die Fettsäuren der Cholesterinester Phosphatide und Triglyceride sowie der unveresterten Fettsäuren bei Gesunden und bei Arteriosklerotikern *Klin Wschr* 38 739 1960

47 Schwartz O H Soule S D & Dunie B Blood lipids in pregnancy *Amer J Obstet Gynec* 39 203 1940

48 Snedecor G W *Statistical methods* Iowa State College Press Ames Ia 1957

49 Sobel E Bossak E T Wang C I & Adlersberg D Serum components in the newborn *Science* 123 461 1956

50 Sprecher H & Robertson A Analyses of lecithin and cephalin fatty acids from human maternal and fetal blood perfused placenta chorion laeve and amnion *J Lab Clin Med* 70 489 1967

51 Svanborg A & Svennerholm L Plasma total lipid cholesterol triglycerides phospholipids and free fatty acids in a healthy Scandinavian population *Acta Med Scand* 169 43 1961

52 Svennerholm L Isolation of gangliosides *Acta Chem Scand* 17 239 1963

53 — Distribution and fatty acid composition of phosphoglycerides in normal human brain *J Lipid Res* 9 570 1968

54 Sweeney M J Etteldorf J N Dobbins W T Somervill B Fischer R & Ferrell C Dietary fat and concentrations of lipid in the serum during the first six to eight weeks of life *Pediatrics* 27 765 1961

55 Ways P & Hanahan D J Characterization and quantification of red cell lipids in normal man *J Lipid Res* 5 318 1964

56 Williams J H Kuchmak M & Witter R F Fatty acids in phospholipids isolated from human red cells *Lipids* 1 391 1966

57 Woodruff C W Bailey M C Davis J T Rogers N & Coniglio J G Serum lipids in breast fed infants and in infants fed evaporated milk *Amer J Clin Nutr* 14 84 1964

58 Zee P Lipid metabolism in the newborn I Phospholipids in cord and maternal sera *Pediatrics* 39 82 1967

59 Zöllner N Wolfram G Londong W & Kirsch K Untersuchungen über die Plasmalipoide des Neugeborenen Säuglings und Kleinkindes *Klin Wschr* 44 380 1966

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infants and their mothers *Amer J Clin Nutr 4* 117 1956
5 Boyd E M Lipid composition of blood in new born infants *Amer J Dis Child 52* 1319 1936
6 Brody S & Carlsson L A Plasma lipid con centrations of the newborn with special reference to the distribution of the different lipid fractions *Clin Chim Acta 7* 694 1962
7 Brown D F McGandy R B Gillie E & Doyle T Observations on some serum compo nents in mothers and in their newborn infants *Amer J Obstet Gynec 77* 556 1969
8 Carlsson L A Determination of serum glyceri des *Acta Soc Med Upsal 64* 208 1959
9 Cramér K & Vikrot O Plasma phospholipid levels and distribution in human maternal blood and in blood from the umbilical cord *Acta Med Scand 179* 1 1966
10 Cravioto J De Licardie E R & Birch H G Nutrition growth and neurointegrative develop ment An experimental and ecologic study *Pe diatrics 38* 319 1966
11 Crawford N An improved method for the de termination of free and total cholesterol using the ferric chloride reaction *Clin Chim Acta 3* 357 1958
12 Crowley J Ways P & Jones J W Human fetal erythrocyte and plasma lipids *J Clin In vest 44* 989 1965
13 Dannenburg W A Burt R L & Leake, N H Plasma lipids in the early puerperium *Amer J Obstet Gynec 84* 1091 1962
14 Dannenburg W A & Burt R L The effect of insulin and glucose on plasma lipids during pregnancy and the puerperium *Amer J Obstet Gynec 92* 195 1965
15 van Deenen L L M Phospholipids and bio membranes *Progress in the Chemistry of Fats and other Lipids 8* 1965
16 Dodge J T & Phillips G B Composition of phospholipids and of phospholipid fatty acids and aldehydes in human red cells *J Lipid Res 8* 667 1967
17 Farquhar J W & Ahrens E H Effects of dietary fats on human erythrocyte fatty acid pattern *J Clin Invest 42* 675 1963
18 Fulco A J & Mead J F Metabolism of essen tial fatty acids VIII Origin of 5 8 11 eicosa trienoic acid in the fat deficient rat *J Biol Chem 234* 1411 1959
19 Gordon M B & Cohn D J Cholesterol and lipoid phosphorus in infancy and in childhood *Amer J Dis Child 35* 193 1928
20 Graven S N Manning G Sagerson R & Rikkers H A study of phospholipids in neonatal hyperbilirubinaemia *J Pediat 66* 12 1965
21 Gyorgy P Über den Lipoidgehalt des Nabel schnur Blutserums und des mutterlichen Blutse rums *Klin Wschr 3* 483 1924
22 Hadnagy C S Lorincz E A Takács T Boaru R P Kifor I Erdélyi A Püpárá I & Róna M Lipid contents of the newborn s blood *Biol Neonat 5* 390 1963
23 Hagerman D D & Villee C A Transport func tions of the placenta *Physiol Rev 40* 313 1960
24 Hallgren B Stenhagen S Svanborg A & Sven nerholm L Gaschromatographic analysis of the fatty acid composition of the plasma lipids in normal and diabetic subjects *J Clin Invest 39* 1424 1960
25 Hansen A F Wiese H F Adam D J D Boelsche A N Haggard M E Davis H Newson W T & Pesut L Influence of diet on blood serum lipids in pregnant women and new born infants *Amer J Clin Nutr 15* 11 1964
26 Herman E & Neumann J Über den Lipoidge halt des Blutes normaler und schwangerer Frauen sowie neugeborener Kinder *Biochem Z 43* 47 1912
27 Hershfield M & Nemeth A M Plasma FFA in mother and fetus Placental transport of pal mitic and linoleic acid in the guinea pig *Fed Proc 26* 2 846 1967
28 Holman R T Caster W D & Wiese H F The essential fatty acid requirement of infants and the assessment of their dietary intake of linoleate by serum fatty acid analysis *Amer J Clin Nutr 14* 70 1964
29 Hornung R Die osmotische Resistenz der Eryth rozyten und der Lipoidgehalt des Blutserums bei Mutter und Kind *Deutsch Med Wschr 44* 1849 1926
30 Jacina J Komorna M Mathéová E & Tisch ler V Die Gesamtlipide der Lipoiden Phos phor das freie und esterfizierte Cholesterol im Serum der Mutter und im Nabelschnurblut *Zbl Gynaek 23* 902 1961
31 Jatzkowitz H & Mehl E Zur Dunnschicht Chromatographie der Gehirn Lipoide ihrer Um und Abbauprodukte *Hoppe Seyler Z Physiol Chem 320* 251 1960
32 Kaplan A & Lee V F Serum lipid levels in infants and mothers at parturition *Clin Chim Acta 12* 258 1965
33 Kleeberg J Lipid determinations in maternal and foetal blood *J Obstet Gynec Brit Comm 70* 701 1963
34 Konttinen A & Pyorala T Serum lipid pattern in normal pregnancy and pre-eclampsia *J Obstet Gynec Brit Comm 71* 453 1964
35 Mendez J Savits B S Flores M & Scrimshaw N S Cholesterol levels of maternal and fetal blood at parturition in upper and lower income groups in Guatemala City *Amer J Clin Nutr 7* 595 1969
36 Miescher K Über Steroide 49 Mitteilung Über Farbreaktionen *Helv Chim Acta 29* 743 1946
37 Mohrhauer H & Holman R T The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver *J Lipid Res 4* 151 1969
38 Mortimer J C Cord blood lipids of normal in fants and infants of diabetic mothers *Arch Dis Child 39* 334 1964
39 O Brien J S & Blankenhorn P H Fatty acid

Table 1 *Regression equations of vital capacity and expiratory flow rates against height*

Dependent variable	Equation	r
FVC water spirometer (litre)		
Male	0 113 e 0 02111 ht	0 925
Female	0 145 e 0 01986 ht	0 942
FVC wedge spirometer (litre)		
Male	0 124 e 0 02072 ht	0 909
Female	0 155 e 0 01944 ht	0 936
MMEF water spirometer (litre sec)		
Male	0 06063 ht − 5 9355	0 799
Female	0 05247 ht − 4 8715	0 770
V_{50} wedge spirometer (litre sec)		
Male	0 06913 ht − 6 8291	0 754
Female	0 05648 ht − 4 96764	0 669
EPFR wright peak flow meter (litres/sec)		
Male	0 09530 ht − 8 40512	0 878
Female	0 10100 ht − 9 20782	0 864
V_{max} wedge spirometer (litres sec)		
Male	0 08024 ht − 6 6184	0 796
Female	0 08278 ht − 7 02453	0 811

three distinct flow volume curves stored on the oscilloscope screen was taken with an oscilloscope camera with adaptor For calculation the EFVC with the highest VC was recorded Peak expiratory flow (V_{max}) and the maximal flow rate at the 50% point of the FVC (V_{50}) on the flow volume curve were calculated

The FEV marker was initially set to start the one second interval when the flow exceeded 40 ml sec Some of the subjects pretriggered the marker generator by unsteady breath holding This caused a discrepancy between the FEV on the flow volume curve and the FEV calculated from the UV recorder on the same breath Decreasing the sensitivity of the marker generator to 100 ml/sec eliminated this discrepancy without significantly changing the FEV

In order to ensure experimental consistency all instruments were calibrated daily The oscilloscope was calibrated in both the abscissa and the ordinate by means of an internal calibration signal For calibration of the wedge and the UV recorder we used a graduated 1 litre syringe for volume and a rotameter calibrated at 1 litre/sec for flow

The expiratory peak flow rate (EPFR) was recorded on Wright's peak flow meter with the subject standing and repeating the performance until the highest possible flow rate was obtained

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Model 197A Hewlett Packard Co Colorado Springs Colo USA

Model 10A1077A Fisher Porter

RESULTS AND DISCUSSION

1 *Normal children*

The regression equations for the forced vital capacity and flow rates plotted against standing height performed with the wedge and water spirometer are given in Table 1

The FVC and the FEV_1 measured by the two instruments were not significantly different (Figs 1 and 2)

V_{50} with the wedge spirometer was found to be significantly higher than the MMEF when performed with the water spirometer ($p < 0.001$) However the MMEF using the wedge spirometer and recorded with the UV recorder on the same breath as the V_{50} was the same as MMEF using the water spirometer There was a good correlation between V_{50} and height This is also true for MMEF (Table 1)

Figs 3 and 4 show the relationship between V_{50} and MMEF and the degree of correction required between these two flow rates

The V_{max} measured with the wedge and the

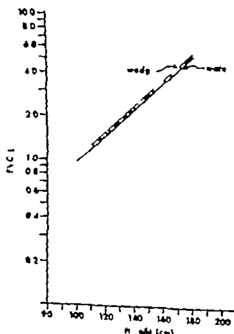


Fig 1 A comparison in healthy children between FVC measured with the water spirometer (FVC = 0 118 e^(0 0… ht)) solid line and the wedge spirometer FVC = 0 1235 e^(0 0… ht)) dashed line

EXPIRATORY FLOW RATES DETERMINED BY WEDGE AND WATER SPIROMETER IN CHILDREN AND YOUNG ADULTS

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Pulmonary function tests are being used with increasing frequency in the investigation and diagnosis of pulmonary disease in children One of the commonest of the routine pulmonary function tests is the forced vital capacity (FVC) manoeuvre The flow volume technique was first described by Hyatt et al (4) and the theory of the relationship of flow volume and trans pulmonary pressure was later elaborated by these same authors (2 3) thus providing the basis for the study of the expiratory flow volume curve (EFVC)

It has been suggested (7) that the EFVC as measured by the waterless wedge spirometer will replace the more common FVC manoeuvre as performed with the standard water spirometer as a routine pulmonary function test

Normal values for children have been reported for the parameters of the FVC test using the standard water spirometer (5 8) There have been no normal values published for the EFVC using the wedge spirometer Measurements of the parameters of the EFVC have been published for children using a volume displacement plethysmograph (9) and by a method which incorporates a pneumotacograph and a light weight spirometer (6) These latter two techniques require sophisticated equipment The wedge spirometer is simple in design and requires little training in its use

The purpose of the study was to compare flow measurements obtained using a wedge spirometer with those obtained using a water spirometer in both healthy and asthmatic children and young adults

MATERIALS AND METHOD

Eighty two subjects (40 males 42 females) aged 6 to 18 years ranging in height from 111 5 cm to 176 5 cm and with no history of cardio-pulmonary disease were studied Fourteen symptomatic asthmatics (5 males 9 females) aged 8 to 15 years were also studied

Water spirometry was performed with a standard 9 litre water spirometer[1] EFVC forced expiratory volume in 1 sec (FEV_1) and maximal mid expiratory flow (MMEF) were measured while the subject performed a forced vital capacity manoeuvre Each manoeuvre was performed with the subject standing and was repeated three times the record with the highest value for FVC being selected

Wedge spirometry was performed with a 570 wedge spirometer[2] The standing subject was connected by a large diameter tube to the spirometer with the electrical outputs connected to an oscilloscope[3] and an ultra violet recorder (UV recorder)[4] A flow triggered one second marker generator developed in this laboratory was connected to the oscilloscope so that the flow volume curve was displaced by 1 cm perpendicular to the volume axis for a 10 m/sec interval 1 sec after initiation of flow As the subject repeatedly performed the forced vital capacity manoeuvre a picture of

Supported by a grant from the Canadian Cystic Fibrosis Foundation

1 Warren & Collins Braintree Mass USA
2 Mod 1370 Med Science Electronics St Louis Mo USA
3 Model 564B Tektronix Inc Beaverton Ore USA
4 S E Laboratories Ltd Feltham Middlesex England

ble 2 *Comparison of V_{50} and V_{max} values in rent and previously reported studies*

udy	Subject's height 130 cm		Subject's height 170 cm	
	V_{50} (litres/sec)	V_{max} (litres/sec)	V_{50} (litres/sec)	V_{max} (litres/sec)
apletal et al (9)				
Male	2 48	3 49	4 65	6 72
Female	2.45	3 19	4 25	5 83
DeMuth et al				
Male	1 75	3 07	2 82	6 42
Female	1 79	67	2.96	5 77
Present study				
Male	2.16	3 81	4 92	7 02
Female	2.37	3 74	4 63	7 05

comparable to the FVC measured by the water spirometer In normal subjects we found the V_{50} to be greater than MMEF and in asthma tics to be less This cannot be accounted for by a change in lung volume since the vital capacity was unchanged suggesting that V_{50} is a more sensitive index of airway obstruction than MMEF The fact that the V_{50} is consistently higher than the MMEF in normal children is due not to the instrument in question, but to the fact that the MMEF is a mean flow over a wide range whereas V_{50} is an instantaneous measure of flow

This finding in symptomatic asthmatic patients agrees with those of Stout et al (7) in adult patients with chronic obstructive pulmonary disease

The EFVC technique using the wedge spirometer is as simple to perform as the FVC manoeuvre with the water spirometer and easier to calculate also the shape of the curve obtained using the wedge spirometer may be in itself diagnostic of obstructive or restrictive lung disease (6) Another advantage of the EFVC is that it includes a measurement of peak flow and can be modified to show a time volume relationship However the equipment is expensive and its electrical calibration must be checked for both flow and volume to insure consistent results

A comparison of the V_{50} and V_{max} predicted for heights of 130 and 170 cm respectively for the present study and those of Zapletal et al (9) and De Muth et al (1) are given in Table 2 The values of V_{50} predicted for the present study are much closer to Zapletal's than DeMuth's but the results for V_{max} in the three studies are reasonably close The main differences between the three studies are probably due to differences in methods

SUMMARY

A comparison between measurements made with a conventional water spirometer and those with a wedge spirometer on 82 healthy normal subjects from 6 to 18 years of age and from 111 5 to 176 5 cm in height was made Fourteen children with symptomatic asthma were also studied It was found that the FVC and FEV_1 were similar on both instruments The values for V_{50} and MMEF were different because V_{50} is an instantaneous measurement whereas MMEF is a mean obtained over a wide range of lung volumes

It is suggested that V_{50} may be a more sensitive index of airway obstruction than the regular MMEF measurement

REFERENCES

1 DeMuth G R Howatt W F & Hill B M The growth of lung function *Pediatrics* 35 Suppl 161 1965

2 Fry D L Theoretical considerations of the bronchial pressure flow volume relationships with particular reference to the maximum expiratory flow volume curve *Phys Med Biol* 3 174 1958

3 Fry D L & Hyatt R E Pulmonary mechanics A unified analysis of the relationship between pressure volume and gasflow in the lungs of normal and diseased human subjects *Amer J Med* 29 672 1960

4 Hyatt R E Schilder D P & Fry D L Relationship between maximum expiratory flow and degree of lung inflation *J Appl Physiol* 13 331 1958

5 Kamel M Weng T R Featherby E A Jackman W S & Levison H Relationship of mechanics of ventilation to lung volumes in children and young adults *Scand J Resp Dis* 50 125 1969

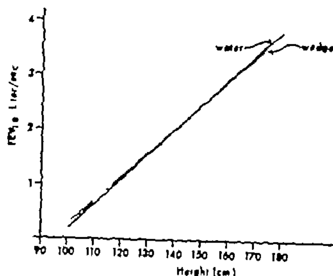


Fig 2 A comparison in healthy children between FEV_1 measured with the water spirometer (FEV = 4 388 + 0 0457 × ht) solid line and the wedge spirometer (FEV = 4 199 + 0 0445 × ht) dashed line

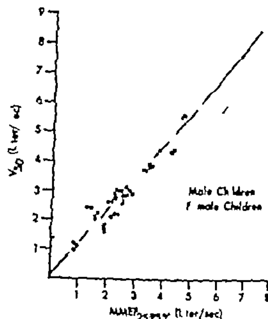


Fig 4 The relationship in healthy children between MMEF measured with the water spirometer and V_{50} measured with the wedge spirometer (V_{50} = 1 143 × MMEF + 0 035) Mean regression line with 95 confidence limits is shown

EPFR measured with the Wright peak flow meter were not significantly different (0 6 > p > 0 5 paired t)

2 *Asthmatic children*

When V_{50} was plotted against MMEF for the 14 asthmatic patients the regression line was calculated to be V_{50} = 1 11 × MMEF—0 16 (r = 0 81) (Fig 5) This regression line gives a lower value for V_{50} for the corresponding MMEF when compared to the regression line for the healthy children V_{50} = 1 143 × MMEF + 0 035 (r = 0 95)

From the above results it is clear that the FVC as measured by the wedge spirometer is

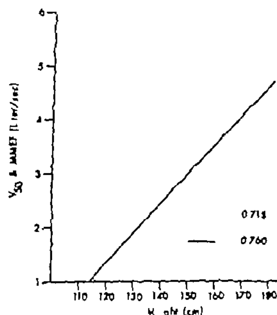


Fig 3 Regression in healthy children of MMEF measured with the water spirometer (MMEF = 0 057 × ht —3 43 r = 0 76) solid line plotted against height and that of V measured with the wedge spirometer (V_{50} = 0 063 × ht − 5 942 r = 0 716) dashed line plotted against height

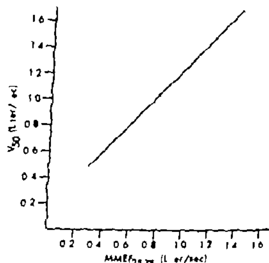


Fig 5 Solid line represents the regression line of V_{50} plotted against MMEF in normal children the dashed line represents the regression line of V_{50} plotted against MMEF for the 14 symptomatic asthmatics

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RESPIRATORY QUOTIENT AND METABOLIC RATE IN NORMAL FULL TERM AND SMALL FOR DATE NEWBORN INFANTS[1]

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The reports concerning the respiratory quotient (RQ) during the first week of life generally agree that the RQ begins to fall shortly after birth but rises again a few days later (4 7 15 16 31) Recent studies have shown a rise in the level of free fatty acids (FFA) glycerol and ketone bodies in the blood of newborn infants during the first few hours after birth (2 11 23 26 28 32 36) Likewise the amount of FFA and the amount and rate of release of glycerol in the newborn's adipose tissue are higher on the first postnatal day (33) On the other hand at the same time the carbohydrate reserve and the blood glucose level decrease (6 39 40) Thus endogenous fat seems to be mobilized after birth indicating increased use of lipids for energy production

Some newborn infants have reduced (premature small for date) or greater (babies of diabetic mothers) carbohydrate and fat reserves After birth the blood level of glycerol and ketone bodies rises rapidly to higher values in premature and small for-date infants than in full term ones (27)

We have thought that a study of postnatal changes in the RQ of small for-date and normal newborn babies might also show from another view point possible differences in energy metabolism

[1] Aided by grants from the Belgian University Fund the Belgian Scientific Research Fund the Swedish Medical Research Council grant B67 19X 17403 and the Association for the Aid of Crippled Children New York

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MATERIAL AND METHODS

Material

Eight normal and full term and six small for-date newborn infants were studied several times from delivery to the end of the first week of life The normal full term babies had a mean birth weight of 3 4 kg and a gestational age between 39 and 42.5 weeks The small for-date infants had a mean birth weight of 2 kg and a gestational age between 36 and 40 weeks When plotted on the Engstrom & Sterky birth weight gestational age chart (12) all of the small for-date babies fell below the tenth percentile line (Fig 1)

Methods

Respiratory metabolism was measured by an open circuit method using the Kipp diaferometer The baby was enclosed in a double Perspex box (22) The air which flowed between the two perspex layers toward the foot end was pumped at a known constant rate (3 85 or 8 1/min) through the gas analyser The chamber was placed inside an Isolette incubator Both oxygen uptake and carbon dioxide production were measured and recorded by a Kipp channel selector and a potentiometric Micrograph Each determination corresponds to 3 hours of recording Activity was continuously monitored by constant observation and graduated by using the arbitrary scale of Oliver & Karlberg (34) Only score O infant asleep and not moving or score I infant asleep moving one extremity or baby with open eyes but physically quiet were accepted for determination of the RQ and the basal oxygen consumption The babies were usually examined between two meals and received breast milk after 12 hours of life No drugs were given

Temperatures were measured and recorded (Philips) by multiple thermocouples These were routinely placed on the abdominal skin in the air stream above the baby's feet at the head-end near the exhaust

6 Lord G P Gazioglu K & Kaltreider N The maximum expiratory flow volume in the evaluation of patients with lung disease A comparative study with standard pulmonary function tests *Amer J Med 46* 72 1969
7 Stout, B D Wiener L Dery D W & Cox J W Clinical application of the flow volume loop *Dis Chest 55* 101 1969
8 Weng T R & Levison H Standards of pulmonary function in children *Amer Rev Resp Dis 99* 879 1969
9 Zapletal A Motoyama E K Van De Woestijne K P Hunt V R & Bouhuys A Maximum expiratory flow volume curves and airway conductance in children and adolescents *J Appl Physiol 26* 308 1969

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Key words Wedge water spirometer respiratory flow rates

DISCUSSION

Protein metabolism apparently contributes little to the energy requirement during the early neonatal period McCance & Strangeways have shown that protein catabolism provides less than 4 of the energy during the first 48 hours of life whereas in adults basal calories are derived up to about 17% from protein catabolism (25) Thus a fall of the RQ values shows acceptable evidence of fat utilization being substituted for carbohydrate utilization as a source of calories However the RQ value in the newborn infant must be cautiously interpreted

First a difference between carbon dioxide output and production may exist over a short period of time (13) From continuous records during several hours wide variations of the RQ have been observed in the present material The RQ values are high in a period of activity and fall steeply in the early ensuing sleep (Fig 5) This could be explained chiefly by unsteady ventilation with drop of the blood CO_2-tension during activity and a recovery during sleep As a significant increase of the blood lactate has been observed in newborns *after a period of activity* (14) an oxygen debt could explain low RQ values during the recovery period High RQ values in muscular activity might also be a consequence of increased carbohydrate catabolism in the muscles

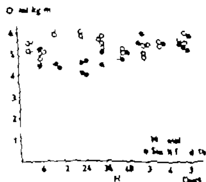


Fig 4 Values of oxygen consumption in small for date and normal birth weight infants during the first 5 days of life

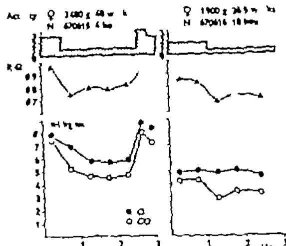


Fig 5 CO_2 production O_2 uptake and RQ values observed in a period of activity (2 or 3) and in the following quiet period (0 or 1) in normal (left) and small for date (right) newborn babies Observe a steady O_2 consumption in the small-for-date infant

After half an hour of sleep a steady state of RQ was usually achieved However rather often slight variations of CO_2 production have been noticed without significant changes in oxygen consumption Sometimes the CO_2 production tends to decrease progressively and then suddenly rises to a higher and new steady state value This is likely related to eye movements and modification of the ventilation during sleep (10 35) Secondly the RQ falls with increasing oxygen consumption in response to cold (5 8 21 22 34 42) During the observation periods the infant was maintained in a neutral range of environmental temperature Moreover as the *radiant* heat loss from the skin to the cold walls of the incubator is at least as important as *the* convective heat loss (30) such losses were prevented by controlling the ambient temperature and by using a double perspex chamber (16) The temperature gradient on both sides of the chamber is less than 1 C thus condition preventing radiant heat loss

A systematic trend of *the RQ* values in the healthy newborn infant has been described in previous studies (4 7 20 31)

The RQ falls during the first days rises again during the next few days to about 0 86

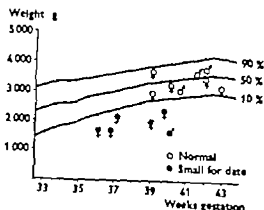


Fig 1 Birth weights of normal and small for-date babies are *plotted in regard to* number of weeks gestation The percentile ranges for female newborn infants according to Engstrom & Sterky (12)

tube and inside the incubator The temperature at *the head end* was considered to be the environmental temperature and was maintained within the *neutral* range according to the Scope chart (37)

Stool and urinary output were carefully observed *As a matter of fact* under our technical conditions the oxygen deflection dropped sharply *when the* chamber within the incubator was contaminated by a *few* drops of urine This is probably due to the evaporation *of the* ammonia *influencing* the gas analyser When such effects were observed *the* chamber was bleaned and the measurement repeated

RESULTS

The RQ values in both groups of newborn babies are plotted with regard to the age in Fig 2 From the statistical analysis of the data two regression lines have been drawn for each group In normal birth weight babies the RQ decreases ($r = -0.63$) significantly ($p <$ 0.05) from the third to the twelfth hour of age From the twelfth hour to the fifth day the RQ values increase ($r = +0.56$) significantly ($p <$ *0.01*) up to a value of 0.87 For the small for date babies the RQ values in the first 12 hours are significantly lower ($p < 0.001$) than in normal birth weight babies and a similar drop is observed From the twelfth hour the RQ values increase ($r = +0.81$) significantly ($p < 0.001$) the regression line is lower ($p < 0.001$) and it slope is steeper ($p < 0.001$) than in the control group The RQ values tend to be similar in both groups as at the end of the first week of life

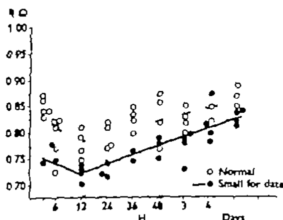


Fig 2 RQ values in both groups of newborn babies are plotted in relation to the age Two regression lines have been drawn for each group one up to 12 hours of age and the other from 12 hours to 5 days

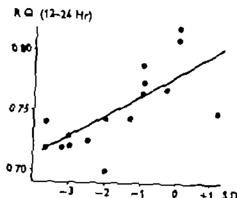


Fig 3 Relationship between the lowest RQ values observed between 12 and 24 hours of life in both groups of newborn babies The differences between actual and predicted weights are expressed as standard deviation units from the mean values according to the intrauterine growth chart

In order to investigate a possible relationship between the degree of small for date and the lowest RQ values, the latter are plotted with regard to the differences in standard deviation units between the actual and the predicted mean values of birth weight according to the birth weight gestational age (12) (Fig 3) The more pronounced the smallness for date, the less was the RQ at the twelfth to twenty fourth hour of life Although the values are scattered the slope of the regression line is statistically significant ($p < 0.001$) and the coefficient of correlation is 0.72 The mean value of oxygen consumption in small for date infants (4.8 ± 0.5 ml/kg/min) is significantly lower ($p < 0.001$) than the normal full term mean value (5.4 ± 0.4 ml/kg/min) (Fig 4)

and rose again during the next few days However the RQ was lower during the first 5 days in small for date infants The O uptake of the small for date babies is initially lower but rises during the first 5 days

Technical problems and clinical implications of the results are discussed

REFERENCES

1 Adamsons K Gandy G M & James L S The influence of thermal factors upon oxygen con umption in the newborn human infant *J Pediat 66* 495 1965

2 Akerblom H Abom T & Somersalo O Acetone bodies in blood of infants and children of various ages *Ann Paediat Fenn 11* 108 1965

3 Auld P A M Bhangananda P & Mehta S The influence of an early caloric intake with i v glucose on catabolism of premature infants *Pediatrics 37* 592 1966

4 Benedict F G & Talbot F B *The physiology of the newborn infant Character and amount of the catabolism* Publ 233 Carnegie Institution of Washington 1915

5 Brück K Temperature regulation in the newborn infants *Biol Neonat 3* 65 1961

6 Cornblath M Segal S & Smith C A Carbohydrate and energy metabolism in the newborn An international exploration *Pediatrics 39* 582 1967

7 Cross K W Tizard J P M & Trythall D A H The gaseous metabolism of the newborn infant *Acta Paediat Scand 46* 265 1957

8 Dawkins M J R & Scopes J W Non shivering thermogenesis and brown adipose tissue in the human newborn infant *Nature 206* 201 1965

9 Dechterm R K Wilkinson R H Davies P A & Ainsworth P Plasma glucose levels in infants weighing 500 g and less fed immediately after birth with breast milk *Biol Neonat 11* 29 1967

10 Dreyfus Brisac C & Monod N Sleep of premature and fullterm neonates A polygraphic study *Proc Roy Soc Med 58* 6 1965

11 Van Duyne C M & Havel R J Plasma unesterified fatty acid concentration in foetal and neonatal life *Proc Soc Exp Biol Med 102* 599 1959

12 Engstrom L & Sterky G Standardkurvor for vikt och langd hos nyfodda barn *Lakartidningen 63* 49_ 1966

13 Farhi L E & Rahn N Gas stores of the body and the unsteady state *J Appl Physiol 7* 472 1955

14 Graven S N Criscuolo D & Holcomb T M The effect of activity on concentration of blood lactate in newborn infants *Pediatrics 37* 278 1966

15 Hasselbalch K A Respirations forsög paa nyfødte born *Bibl Laeg 5* 219 1904

16 Hey E N & Mount L E Heat losses from babies in incubators *Arch Dis Childh 42* 75 1967

17 Hill J R & Rahimtulla K A Heat balance and the metabolism rate of the newborn babies in relation to environmental temperature and the effect of age and of weight on basal metabolism rate *J Physiol 180* 239 1965

18 Hill Jane R & Robinson D C Oxygen consumption in normally grown small for-dates and large for-dates new born infants *J Physiol 199* 685 1968

19 Jonxis J H P Van der Vlugt J J De Groot C J Boersma E R & Meijers E D K *The metabolic rate in premature dysmature and sick infants in relation to environmental temperature* Nutricia Symposium H E Stenfert Kroese Leiden Holland 1968 pp 201 209

20 Karlberg P Determinations of standard energy metabolism (basal metabolism) in normal infants *Acta Paediat Scand 41* Suppl 89 1952

21 Karlberg P Moore R E & Oliver T K Jr The thermogenic response of the newborn infant to noradrenaline *Acta Paediat Scand 51* 284 1962

22 — Thermogenic and cardiovascular responses of the newborn baby to noradrenaline *Acta Paediat Scand 54* 225 1965

23 Keele D K & Kay J L Plasma free fatty acid and blood sugar levels in newborn infants and their mothers *Pediatrics 37* 597 1966

24 Levison H & Swyer P R Oxygen consumption and the thermal environment in newly born infants *Biol Neonat 7* 305 1964

25 McCance R A & Strangeways W M B Protein catabolism and oxygen consumption during starvation in infants young adults and old men *Brit J Nutr 8* 21 1954

26 Melichar V Drahota Z & Hahn P Changes in the blood levels of acetoacetate and ketone bodies in newborn infants *Biol Neonat 8* 348 1965

27 — Ketone bodies in the blood of full term newborns premature and dysmature infants and infants of diabetic mothers *Biol Neonat 11* 23 1967

28 Melichar V & Wolf H Postnatal changes in the blood serum content of glycerol and free fatty acids in premature infants Influence of hypothermia and of respiratory distress *Biol Neonat 11* 50 1967

29 Mestyan J Fekete M Bata G & Jarai I The basal metabolic rate of premature infants *Biol Neonat 7* 11 1964

30 Mount L E Basis of heat regulation in homeotherms *Brit Med Bull 22* 84 1966

31 Murlin J R Conklin R & Marsh E Energy metabolism of normal newborn babies with

This trend has also been observed here, however the RQ does not fall so deeply and thereafter rises earlier than previously reported These differences call for two comments The lowest RQ values previously published could be due to an environmental temperature lower than the neutral range The earlier rising of the RQ values is likely related to the earlier feeding of the newborn in former investigations, the RQ values rise after the second day and the increase coincides with the milk feeding In the present study milk feeding was started at the twelfth hour of age

Considering the RQ differences between small for date and normal infants and despite a wide scattering of the values in both groups, the RQ is lower during the first 5 days in the small for date infants These results could be usefully considered in relation to recent reports on lower blood glucose and carbohydrate storage in foetal malnutrition (3 6, 9 39 40, 43) Moreover FFA glycerol and ketone bodies in the blood rise rapidly to higher values in the small for date infants than in normal full term infants (27) hence lipids are mobilized and utilized to a greater extent in the former group Oxygen consumption values in these two small groups are within the range of wider published investigations (1 17 24 34) The mean value of O uptake in small for date infants is lower than in full term babies According to the report of Sinclair et al (41) a higher value of oxygen consumption would be expected in small for-date infants their extracellular fluids being probably diminished On the contrary our results of oxygen consumption in small for-date infants are similar to the values in premature babies (29 38) There is also the possibility of oxygen consumption decreasing as a starvation reaction from foetal malnutrition In the small for date group oxygen consumption rises with age ($r = 0\ 58$) ($p < 0\ 001$) Contrarily oxygen consumption does not change significantly ($r = 0\ 18$ $p = 0\ 28$) in normal fullterm infants During the writing of this paper Jonxis et al published extensive results of O uptake and RQ values in normal full term premature and small for date infants in relation to environmental temperature (19) In all three groups the RQ dropped during the first day of life and rose again from the third day onwards On the third day of life the RQ was respectively 0 80 in full term, 0 78 in premature and 0 75 in small for date infants Especially in the small for-date group, values close to 0 70 have been observed In all groups, the RQ rises when larger quantities of food are given but feeding was started only on the second day of life In many small for date babies there is a marked drop in the RQ combined with an increased O_2 consumption during exposure to cold The O uptake of the small for-date babies was initially 4 7 ml/kg/min and rose to 6 8 ml during the first 10 days This agreement between the results of two independent studies which could be expected theoretically is encouraging when actually observed

However the results obtained in these studies do not correspond with the oxygen consumption values in normally grown small for-date and large for date newborn infants in the recent study of June Hill & Robinson (18) They found no significant differences between these groups in agreement with the study of Sinclair et al (41) The differences might be of methodological origin Since Hill & Robinson measured the oxygen consumption over a period of approximately 10 min at a number of different environmental temperatures and also in cold conditions during an interval between 3 hourly feeds but we determined our results after half an hour of sleep the steady state might have been more pronounced in the present study The heat loss might also have been kept at a lower level According to Jonxis et al the small for date infants show a more pronounced metabolic response to a cold environment

SUMMARY

Respiratory quotient and gaseous metabolism were measured in normal full term and small for-date infants during the first week of life In both groups RQ dropped during the first day

THE EFFECT OF DIURETICS IN LATE PREGNANCY ON THE NEWBORN INFANT

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The mechanism for the distribution of water between the mother and the foetus is not precisely known Several investigators have found that administration of hypo- and hypertonic solutions intravenously to the pregnant mother has a prompt effect on the total osmotic pressure and the concentration of electrolytes in the foetus Changes in the extracellular compartments of the mother are also accompanied by rapid changes in the fetal extracellular space (1 12)

From a theoretical point of view it could be expected that administration of diuretics during the last part of pregnancy might have some effect on the water balance of the newborn infant indirectly by removing water and electrolytes from the mother or directly have an effect in cases where the diuretics freely pass the placenta barrier as e g the thiazid group has been shown to do (4)

Since the introduction in 1957 of the thiazides they have been widely used in the prophylaxis and treatment of toxemia of pregnancy and in the treatment of oedema in pregnancy (11 13 15) In the available literature however there has been no reports of disturbances in the body water compartments or in the mineral metabolism of the newborn infant

The aim of the present study was to investigate if the administration of the two diuretics of the thiazid group Bendroflumenthazid and Chlortalidone during the last part of pregnancy until delivery has any detectable effect on the water and mineral metabolism in the newborn infant

This work has been supported by grants from the Danish Medical Research Council and the D Marshall Foundation

MATERIAL

This comprises 16 infants a control group of 8 full term infants born of normal mothers and a group of 8 full term infants born of mothers who had been treated with Bendroflumenthazid or Chlortalidone during a various length of time until delivery In all cases the diuretic was given until the day of delivery In the treated group there was an additional administration of potassium salt The indication for the diuretical treatment was abnormal weight gain and oedema in no cases toxemia was present The type of drug the dose and the duration of administration is shown in Table 1 There was no sign of dehydration

Within a few hours before or after delivery blood samples were drawn from the mother for determination of sodium potassium and chloride concentration as well as total osmolality Preliminary observations have shown that these values vary very little during the hours before and after delivery In the newborn infants the total body water TBW the extracellular water ECW the concentrations of sodium potassium and chloride and the osmolality was determined 2–4 hours after birth (In one case 1620/69 the determination was made 9 hours after birth)

The values in the 2 groups were compared

METHODS

The total body water was determined by the dilution method using deuterium oxide D O 99 78 g/100 g

special reference to the influence of food and of crying *Amer J Dis Child* 29 1 1925
32 Novák M Melichar V & Hahn P Postnatal changes in the blood serum content of glycerol and fatty acids in human infants *Biol Neonat* 7 179 1964
33 — Changes in the content and release of glycerol in subcutaneous adipose tissues of newborn in fant *Biol Neonat* 8 253 1965
34 Oliver T K Jr & Karlberg P Gaseous metab olism in newly born human infants The effects of environmental temperature and 15 oxygen in the inspired air *Amer J Dis Child* 105 427, 1963
35 Parmelee A H Bruck K & Bruck M Activity and inactivity cycles during the sleep of prema ture infants exposed to neutral temperature *Biol Neonat* 4 317 1962
36 Persson B & Gentz J Free fatty acids and glycerol in plasma of newborn infants *Acta Paediat Scand* 54 198 1965
37 Scopes J W Metabolic rate and temperature control in the human baby *Brit Med Bull* 22 88 1966
38 Scopes J W & Ahmed I Minimal rates of oxygen consumption in sick and premature new born infants *Arch Dis Childh* 41 407 1966
39 Shelley H J Glycogen reserves and their changes at birth and in anoxia *Brit Med Bull* 17 137 1961
40 Shelley H J & Neligan G A Neonatal hypo glycemia *Brit Med Bull* 22 34 1966
41 Sinclair J C Scopes J W & Silverman W A Metabolic reference standards for neonate *Pe diatrics* 39 724 1967
42 Silverman W A & Agate F J Variation in cold resistance among small newborn infants *Biol Neonat* 6 113 1964
43 Stern L Sourkes T L & Raiha N The role of the adrenal medulla in the hypoglycemia of foetal malnutrition *Biol Neonat* 41 129 1967

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Key words Respiratory quotient metabolic rate full term newborn infants small for date newborn in fants

Table 2*b* *The values of body compartements serum electrolytes and osmolality in infants and in mothers who has been treated with diuretics in late pregnancy*

Case no	Sex	Weight	TBW (%)	ECW (%)	Sodium mEq/l		Potassium		Chloride		Osmolality	
					Inft	Mother	Inft	Mother	Inft	Mother	Inft	Mother
A 1435/69	♂	3 680	76 3	37 4	139	138	4 5	3 9	111	109	287	280
A 1452 69	♀	3 660	73 8	44 5	142	140	4 2	3 6	108	106	280	284
A 1531/69	♂	2 740	75 3	—	138	136	3 9	3 4	107	105	285	271
A 1603/69	♀	3 090	74 2	36 8	145	148	3 7	3 4	111	111	296	307
A 1680 69	♀	3 410	70 8	40 4	140	144	4 5	3 6	101	101	282	282
A 1111/69	♂	3 600	74 0	38 1	140	140	4 4	3 7	105	102	278	285
A 1560 69	♂	4 560	69 7	34 9	139	139	4 8	3 8	103	104	280	287
A 1620/69	♀	3 410	76 7	34 3	144	141	3 9	3 7	103	102	290	290

the osmolality in the mothers and the newborn infants in the untreated control group is shown in Table 2*a* and the values for the group treated with diuretics is shown in Table 2*b* The mean values for the two groups of infants is shown in Table 3 and the mean values for the two groups of mothers in Table 4

The TBW of the infants in both groups was nearly identical The ECW in the treated group was slightly reduced as well as the osmotic and the sodium potassium and chloride concentrations This reduction was not statistically significant using the Student's *t* test

Among the mothers there was a trend towards higher values of sodium concentration and of osmolality and towards lower values for potassium and chloride in the treated group compared to the control group The transplacental difference in sodium concentrations was larger in the control group (3 8 mEq/l) than in the treatment group (0 1 mEq) This difference is significant with a *p* value less than 0 001 using the Student's *t* test

The changes observed in the infants of the treated group were not correlated to the duration of administration of diuretics and no comparison between the two diuretics was possible

DISCUSSION

The values for TBW and ECW in the control infants correspond well to those found by others (4 8 10) The h[illegible]es for the sodium and potassium concentrations as well as for the osmolality in the infants compared to the mothers is similar to the findings of other investigators (7 10 16)

The dominant action of the thiazids is to increase the renal excretion of sodium and chloride and an accompanying volume of water mostly derived from the extracellular compartment This is the result from inhibition of the

Table 3 *The mean values for body compartments serum electrolytes and osmolality in newborn infants from mothers with and without diuretical treatment in late pregnancy*

Mean values	Without diuretics	With diuretics
Weight, g	3 583	3 513
TBW	74 0	73 9
ECW	40 3	38 1
Sodium mEq/l	142 6	140 3
Potassium mEq/l	4 61	4 24
Chloride mEq/l	107 8	106 1
Osmolality mosmol/l	288 6	284 8

Table 4 *The mean values of serum electrolytes and osmolality in mothers with and without diuretical treatment in late pregnancy*

Mean values	Without diuretics	With diuretics
Sodium mEq/l	138 8	140 8
Potassium mEq/l	3 86	3 64
Chloride mEq/l	107	105
Osmolality mosmol/l	282.5	285 8

Table 1 *Dose of diuretics and the number of days before delivery it was administered*

Chlortalidone Hygroton (D) 100 mg Bendroflumethazid Centyl (D) 2 5 mg c KCL 573 mg

Case no	
A 1435/69	Chlortalidone 100 mg in 36 days followed by Bendroflumethazid in 48 days
A 1452/69	Bendroflumethazid 10 mg in 10 days
A 1531/69	Bendroflumethazid 2 5 mg in 20 days
A 1603/69	Bendroflumethazid 5 mg in 28 days
A 1680/69	Bendroflumethazid 2 5 mg in 38 days
A 1111/69	Bendroflumethazid 2 5 mg in 24 days
A 1560/69	Bendroflumethazid 5 mg in 6 days
A 1620/69	Chlortalidone 100 mg in 18 days

supplied in ampoules of 25 g which was transferred to 5 ml ampoules after addition of 9 mg NaCl/ml and sterilized Approximately 1 5 ml D O per kg body weight were given through an umbilical vein catheter with calibrated syringes Equilibration was reached at 2 hours and 2 plasma samples for double determinations were drawn through the catheter at 2 / and 3 hours after injection and stored at − 10 C in sealed tubes each containing 3/4 1 ml plasma Vacuum sublimation of the plasma samples were performed (9) and the concentration of D O in H O was measured in an infrared spectrofotometer (Perkin Elmer 421) by using the absorbance at 3 98 μ (2513 cm − 1) (14 17)

The concentration of D O in H O was in the range of 0 1-0 2 vol % and as standard was used D O in distilled water in the concentrations of 0 1 0 2 0 3 and 0 4 vol %

The procedure for measuring an unknown sample was as follows the 2 standards on either side of the expected value was determined and the concentration of the unknown sample was interpolated

The 2 standards always preceded the sample If more than 3 unknown samples were analyzed a new set of standards was read at the conclusion of the analysis and for the interpolation the average of standards before and after analysis was used

TBW is calculated from the equation

$$T = \frac{\text{injected volume of } D_2O}{\text{conc of } D_2O \text{ in plasma}}$$

No correction was made for the impurity of D O the exchange of labile hydrogen atoms of the solid constituent of the body (9) or for the excretion of D O before equilibrium

The extracellular water was determined by using the thiosulfate volume of dilution in a modification (3) of the micromethod originally described by Newman et al (6) A 5 solution of sodium thiosulfate was used and 1 5 ml/kg body weight were injected through the umbilical vein catheter just before the injection of D O Blood samples for analysis were drawn from the heel and the values 20 30 40 50 and 60 min after injection were determined From these the zero value was calculated using the least square method The ECW was expressed in per cent of total body weight just prior to injection in the same manner as with TBW

The osmolality was determined by an Knauer osmometer and the coefficient of variation on a double reading was 0 33*

RESULTS

The effect of diuretics on the pregnant mother was in all cases shown by a weight loss A side effect of thiazid is a reduced excretion of uric acid and an elevation of the serum uric acid In a small trial the mean value of serum uric acid was 7 2 mg/100 ml in the treated group against 4 2 mg/100 ml in 6 mothers in late pregnancy who had received no treatment with diuretics

The values for body water compartments in the newborn infants and the serum concentrations of sodium potassium and chloride and

* The osmolality was measured at the Medical Physiologic Institute C Rådmandsgade 71 Copenhagen N Head Professor N A Thorn

Table 2*a* *The values of body compartements serum electrolytes and osmolality in the control group*

					Sodium mEq/l		Potassium		Chloride		Osmolality	
Case no	Sex	Weight	TBW ( )	ECW ( )	Inft	Mother	Inft	Mother	Inft	Mother	Inft	Mother
A 1466/69	♂	2 825	79 9	39 3	151	140	4 5	3 8	114	108	324	281
A 1470/69	♀	2 560	79 0	46 1	144	137	4 9	4 0	106	105	285	277
A 1489/69	♂	4 880	67 4	34 8	139	136	4 6	3 8	106	106	277	272
A 1474/69	♂	3 715	72 7	38 0	140	137	4 8	4 2	107	107	290	291
A 1481/69	♀	3 090	81 0	41 7	141	136	4 0	3 3	104	106	290	289
A 1516/69	♂	3 740	73 2	44 0	143	143	4 8	3 9	108	107	282	285
A 1549/69	♂	3 860	70 6	44 9	140	137	4 7	3 9	106	105	275	275
A 1592/69	♀	4 000	68 2	33 7	143	144	4 6	4 0	111	112	286	290

16 Thalme B Electrolyte and acid base balance in fetal and maternal blood Acta Obstet Gynec Scand 45 Suppl 8
17 Turner M D Neely W A & Hardy J D Rapid determination of deuterium oxide in biological fluids Appl Physiol 15 309 1960

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tubular mechanism of electrolyte reabsorption and a well known side effect is hypocalemia

In the mothers of the treated group there was a clinical effect with loss of weight and disappearance of edema From the doses used an effect on the electrolyte concentrations in the mothers blood would not be expected, actually in this study insignificantly higher values for sodium and osmolality were found

In infants born to mothers treated with diuretics some changes in water and mineral metabolism were observed as compared to the control group These observations are not statistically significant, there is however a general trend in all 6 parameters pointing in the same direction TBW was only slightly reduced ECW was somewhat more reduced as well as the concentrations of sodium potassium and chloride and the osmolality These findings could be interpreted as the result of a slight water and salt depletion The statistically confirmed observation that the concentration gradient from infants to mother for sodium and the difference of the osmolality is lessened in the diuretic group is in agreement with this interpretation

The question, if there is 1) an indirect effect via changes in the mother in the water and mineral metabolism caused by the diuretics or 2) a direct action on the placenta or 3) a direct renal effect on the foetus, cannot be precisely answered But the fact, that thiazides are able to pass the placenta barrier and that the changes between the two groups of infants are more marked than the changes between the mother groups could either mean that there is an effect on the kidney of the foetus a direct effect on the placenta, or a combination of the two effects

SUMMARY

The changes in water and mineral metabolism in newborn infants born of mothers, who has been treated in late pregnancy with diuretics, was investigated It was found that all 6 parameters the total body water the extracellular water the serum concentrations of sodium potassium and chloride and the osmolality all pointed in the direction that there was a slight salt and water depletion in the infants from the mothers, treated with diuretics

The biochemical observations were not clinically detectable

REFERENCES

1 Battaglia F Prystowsky H Smisson C Hellegers A & Bruns P The effect of the administration of fluids intravenously to mothers upon the concentration of water and electrolytes in plasma of human fetuses *Pediatrics 25 2 1960*

2 Friis Hansen B The extracellular fluid volume in infants and children *Acta Paediat Scand 43* 444 1954

3 — Body water compartements in children *Pediatrics 28 169 1961*

4 Garnet J D Placental transfer of chlorothiazide *Obstet* Gynec 23 123 1963

5 Gladtke, E Die Bestimmung des extracellularen Flussigkeitraumes bei Kindern mit Hilfe von Thiosulfat *Z Ges Exp Med 141* 301 1966

6 Newman E W Gilman A & Philips F S Renal clearance of thiosulfate in man *Bull Johns Hopk Hosp 79 229 1946*

7 Mellor D J Cockburn F & Lees M M Distribution of ions and electrical potential differences between mother and fetus in the human at term *J Obstet Gynec* 76 993 1969

8 Osler M Body water of newborn infants of diabetic mothers *Acta Endocr 34* 261 1960

9 *Schloerb P R Friis Hansen B* Edelman I S Soloman A K & Moore F D The measurement of total body water in the human subject by deuterium oxide dilution *J Clin Invest 23 1296 1950*

10 Seeds E Water metabolism of the fetus *Amer J Obstet Gynec 92 727 1965*

11 Sele V Benzylhydroflumethiazide (Centyl Leo) virkning på svangerskabsødemer *Ugeskr Læg 122* 1017 1960

12 Seller M J The effects of maternal overhydration on the rat foetus *Quart J Exp Physiol 48 292 1963*

13 Shubeck F & Tatum H Prophylactic use of Chlortalidone in pregnancy *Obstet Gynec 23* 353 1964

14 Stansell M & Mojica L Determination of body water content using trace level of deuterium oxide and infrared spectrophotometry *Clin Chem 14* 1112 1968

15 Tatum *H J & Waterman E A* The prophylactic and therapeutic use of thiazides in pregnancy *Practitioner 24* 101 1961

16 Thalme B Electrolyte and acid base balance in fetal and maternal blood Acta Obstet Gynec Scand 45 Suppl 8
17 Turner M D Neely W A & Hardy J D Rapid determination of deuterium oxide in biological fluids Appl Physiol 15 309 1960

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THREE CASES OF DISSEMINATED INTRAVASCULAR COAGULATION

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Disseminated intravascular coagulation (DIC) should be considered in relation to sudden deterioration in the course of various diseases in childhood as well as with unexplained bleeding conditions. If DIC can be diagnosed and interrupted before extensive thrombosis and tissue damages have occurred a prompt recovery can be obtained.

The mechanism in DIC is an extensive formation of fibrin in the vascular system especially in the small vessels. During the process, coagulation factors are consumed, above all fibrinogen, platelets factor V and factor VIII. There is an increased activation of plasminogen to plasmin which lyses part of the formed fibrin and fibrinolytic split products are released. In the presence of these and thrombocytopenia continued fibrin formation will result in abnormal clotting which has been observed to damage the erythrocytes and thus give rise to hemolytic anemia (6 24).

The sudden loss of coagulability of the blood was first observed and described in obstetrical practice (9). Further investigations demonstrated that in this situation thromboplastic material had entered the maternal circulation (23). The material which initiates the DIC is often amniotic fluid with debris, but tissue fragments and bacterial products can also be responsible (22).

The occurrence of DIC concomitant to various conditions has been much discussed for example in conjunction with hemolytic uremic syndrome, hemolytic anemia malignant diseases sepsis burns snake bites and many other conditions (7 8 12, 13, 16). However there is a striking resemblance between the suddenly developing changes including clotting defects found in some diseases and those changes obtained in experimental animals by injecting thromboplastic material (11). There can be a rapid change in the general condition often accompanied by shock. Definitive diagnosis of DIC can occasionally be made at biopsy otherwise only in patients, who die in an acute phase of the disease, because the thrombi in the small vessels only exist for a short time.

Three cases treated at Children's Hospital, Goteborg are here presented. The clinical findings indicated DIC and quantitative normalization of the clotting factors was obtained as soon as the formation of excessive fibrin was interrupted.

CASE REPORTS

Case 1

59 04 30 Y S A previously healthy 4 year-old girl was admitted to Children's Hospital Goteborg because of abdominal pain and vomiting for 4 days. The abdominal pain had started suddenly and was of intermittent type increasing in severity. On admission she was pale tired and complaining of diffuse pain in the abdomen. She ran a slight fever. Around the neck she had a few petechiae. In the left upper quadrant of the abdomen a solid rounded tumor was palpated extending 6 cm below the arch. Urography demonstrated that the left kidney was pushed down by a tumor but the appearance of the kidney was not otherwise influenced. The laboratory findings

Table 1. *Laboratory findings in three patients with disseminated intravascular coagulation*

Case 1 1. Day of admission. 2, 3 days after operation. *Case 2* 1. At 12 hours of age. 2. At 3 days of age.
Case 3 1. Day before the renal biopsy. 2. Day of deterioration (3 days after biopsy). 3. 3 days later.

	Case 1		Case 2		Case 3		
Investigation	*1*	*2*	*1*	*2*	*1*	*2*	*3*
Fibrinogen mg/100 ml	0	550	110	260	920	90	550
Number of platelets/mm^3	20 000	388 000	80 000	180 000	325 000	60 000	190 000
P & P	26	56	—	—	—	—	—
Prothrombin	—	—	27	45	67	48	79
Factor V	33	80	58	71	96	42	89
Factor VIII *	21	92	54	78	86	28	67
Fibrinolytic activity	—	—	—	—	—	—	—
Fibr. split products	+	—	(+)	—	—	+	—

* Blood collected with Epsilon aminocaproic acid.

(Table 1) *demonstrated a moderate anemia.* No fibrinogen could be demonstrated by electrophoresis or clotting methods (3) but with immunodiffusion analysis a faint fibrinogen precipitate was observed. A moderate decrease of PP value (19), factor V (20) and factor VIII (4) *was observed, but factor IX* (4) was unchanged. *The* number of platelets was diminished. No fibrinolysis could be demonstrated (1) but with immunodiffusion methods (2) fibrinolytic split products were identified in serum. The test of liver function was normal. *In the urine some proteinuria* was noted *occasionally,* but the sediment as well as the osmolarity was normal. X-ray of the skeleton and the lungs did not show metastasis.

During the days following admission the condition of the girl was unchanged, but she developed *a moderately* increased bleeding tendency with nose bleedings and a few subcutaneous haematomas. As the clotting disturbances could have been initiated by the tumor, it was decided to remove it.

Early in the morning of the operation day she obtained 400 ml platelet rich plasma (each ml contained platelets from 10 ml normal blood). 5 hours later she *received preoperatively* 2 g fibrinogen (Kabi, Stockholm) and 400 ml fresh blood. No excessive bleeding occurred during the operation (S. Hagberg) but she was given another 400 ml fresh blood, 1 g fibrinogen and 200 ml platelet-rich plasma and 2 hours after the operation 1 g *epsilon aminocaproic* acid.

A retroperitoneal encapsulated rounded tumor with a diameter of about 12 cm was extirpated after difficulty in freeing it from the surrounding tissues, especially from the aorta and its branches. The tumor had developed from the left suprarenal gland which was also extirpated. The weight of the tumor was almost 800 g. The sectioned surface was dark red and smeary but no solid clots were visible. At microscopic examination it was noted that the tumor consisted of bundles of tumor cells arranged in an alveolaric structure filled with bleedings and vessels. The pathological diagnosis was neuroblastoma of the type called sympathicogonioma tenue fibrillare (J. Mellgren).

In order to determine its clotting action a saline extract of the tumor was *made after removing the* blood by repeated washing. For comparative purposes similar extracts were made from normal suprarenal tissue, brain tissue and tissue from neuroblastomas from other patients. The fibrinolytic activity of all of *these tissue-extracts* was almost none when tested with the fibrin plate method (1). The thromboplastic activity of the different extracts was measured by noting their effect on normal plasma in the recalcification test. The tumor tissue *from the* patient *showed as* potent *thromboplastic* activity as the brain tissue, while the tumor extracts from the other patients as well as the normal suprarenal tissue hardly influenced the recalcification time.

Postoperatively the patient did well and *the* clotting factors *reached normal* levels. Fibrinogen and factor VIII increased to about twice normal values *during the two* subsequent days, whereafter they normalized. Because of the malignancy of the tumor the patient received postoperative radiation treatment as well as cytostatic drugs *for* a period of 14 days. Two years later she was in good health and showed no signs of *relapse* in regard to the tumor or the clotting defects.

Case 2

69.07.22. Girl R. This newborn baby was sent to Children's Hospital directly after delivery because of extensive bleeding from an accidental cut on her face. She was the first child of a healthy *mother.* During the normal delivery the *foetus* developed intrauterine asphyxia and an emergency cesarean section was performed. *During* this procedure a cut in the left parotic region of the infant was accidentally made at the incision of the uterus. The wound was 5 cm long and extended down *to the* bone. It was found necessary to suture the wound. During the following hours a large *hematoma* developed infiltrating the *soft* tissue of the neck. The general

condition of the infant deteriorated she went into respiratory distress and intubation was performed During the next 12 hours the bleeding persisted The infant obtained 600 ml fresh blood to compensate for the blood loss The wound was opened in search of a bleeding vessel None was found but blood was oozing from everywhere A blood sample drawn at that time demonstrated a moderate decrease of pro thrombin (18) factor V and factor VIII and decrease in number of platelets (Table 1) The diagnosis DIC was possible and the child was started on heparin treatment (200 U/kg body weight every 6 hours) Four hours later she was given epsilon aminocaproic acid (0 1 g/kg body weight) The bleeding ceased shortly after the administration of heparin and there was no need for further blood transfusion There after the child recovered quickly and the heparin treatment was discontinued after 2 days 12 hours later the levels of the clotting factors were normal She was taken out of the respirator the tube was removed and 2 weeks later she was completely well

Case 3

56 01 29 G A This girl 12 years of age had prev iously been healthy except for a urinary tract infec tion at the age of 8 in 1964 probably cystitis Four months prior to admission she was ill with moderate fever back pain and bacteriuria which persisted for almost 2 months She had courses of treatment with sulpha tetracycline and nitrofurantoin Urography at that time was normal Two months later she developed signs of renal insufficiency with proteinuria oedema increased non protein nitrogen and hypertension She was admitted to Children s Hospital Goteborg For evaluation of her disease an open renal biopsy was essential Except for a high fibrinogen content the investigation of clotting factors the day previous to the kidney biopsy was completely normal Biopsy was performed without complication The histological diagnosis was sub acute glomerulonephritis with endo thelial and epithelial proliferation in the capillary loops of the glomeruli In the tubuli degenerative changes were demonstrable Three days after the ope ration the condition of the patient suddenly deterio rated and she went into preshock She had contracted a urinary tract infection with *E coli* and enterococci hemolytic anemia and anuria A slight increase in the bleeding tendency was observed She had an oozing bleeding from the operation wound and from punc tures in her fingers after blood tests The coagulation investigation demonstrated a decrease of the prev iously very high fibrinogen content factor V and factor VIII as well as the number of platelets No fibrinolytic activity was noted but fibrinolytic split products were observed with immunodiffusion meth ods (Table 1) She was given plasma transfusion (20 ml/kg body weight) antibiotics and then started on a heparin treatment (125 U/kg body weight every 6 hours) Three days later normalization of the clot ting factors had occurred the hemolysis had disap peared and the amount of urine was increasing The heparin treatment was continued for about 2 months with a short interval when she needed a surgical cleaning of the biopsy wound which demonstrated very poor healing with various bacterial infections After the surgical cleaning it healed well Her primary disease was treated with cortisone and 6-mercaptopurine During the following months she im proved though she continuously demonstrated signs of serious kidney damage Later she went into renal failure and died 6 months afterwards Examination of the kidneys demonstrated the same changes as earlier found at biopsy

DISCUSSION

Of these 3 patients the bleeding tendency do minated the symptoms only in case 2 Deterio ration of the general condition was the main symptom in the others possibly due to the blockage of the microcirculation The deteriora tion was slowly progressive in case 1 and rapid in case 3 These 2 patients also had a slightly increased bleeding tendency which could easily have been overlooked In case 1 the change consisted of a few petechiae and in case 3 ooz ing from the operation wound and finger punc tures after blood tests In all 3 cases the coagu lation pattern showed reduction of prothrom bin, factor V and factor VIII as well as num ber of platelets These changes were most ob vious in case 1 In case 3 the levels were not as strikingly low as in case 1 however in rela tion to the prebiopsy figures a considerable decrease was apparent The very high prebiopsy fibrinogen content can be explained by the re nal disease In case 2 the absolute figures are obscured by the large amount of transfused blood

For practical purposes the diagnosis DIC can be strongly suspected by a low platelet count combined with a decreased level of fibrinogen when one or the other of these findings cannot be related to the basic condition The demon stration of the changes in the other coagulation factors is time-consuming and often requires specialists

Of the differential diagnosis acute thrombo cytopenia and fibrinolysis should be considered In acute thrombocytopenia the fibrinogen level is normal and the platelet count is usually

lower than that in DIC. Values below 20 000/mm³ are common in thrombocytopenia whereas in DIC the decrease is usually not as great. In patients with primary fibrinolysis the bleeding tendency always dominates the symptoms. Moderate increased fibrinolytic activity can sometimes be demonstrated in DIC; in primary fibrinolysis the fibrinolytic activity is pronounced and followed by a decrease of the level of fibrinogen and also of other clotting factors. The number of platelets is however always unaffected.

The initiating cause of the intravascular clotting in these 3 patients varied. In case 1 the tumor demonstrated a very high thromboplastic activity. It is known that malignant tumors can promote intravascular coagulation but there has been much speculation about the exact mechanism. Blix & Jacobsen (5) suggested that thromboplastic material from a tumor was released into the blood stream. In case 1 fragments of the tumor in the blood stream would very probably give rise to a severe intravascular clotting because of the potent thromboplastic activity of this tumor. The contrary action of other neuroblastomic tissue is puzzling.

Case 2 had bleeding tendency immediately after the delivery. The child received the laceration some minutes earlier when it was still in utero surrounded with meconium-containing amniotic fluid. A speculation is that some of the amniotic fluid was sucked into the opened vessels of the foetus. The thromboplastic effect of meconium-containing amniotic fluid is known (22). A contributing factor could be that the infant had intrauterine asphyxia.

In case 3 the renal biopsy did not seem to be the cause of DIC. The urinary tract infection with released endotoxins from *E. coli* could be the trigger mechanism of DIC. The conditions were perhaps particularly favourable for the accessibility of the toxin in the newly biopsied kidney. It might have started as a Shwartzman reaction which is known to be mediated by intravascular clotting (15). Hardaway (11) also states that high levels of blood fibrinogen in pregnancy promote the Shwartzman reaction and in case 3 it was demonstrated that her fibrinogen level was very high.

The recovery of the 3 patients was prompt after interrupting the intravascular coagulation in case 1 through extirpation of the tumor, in cases 2 and 3 through immediate treatment with heparin. In case 3 treatment was continued for almost 2 months with the hope of preventing further local fibrin deposits in the kidney as a result of an antigen antibody reaction (14) in the kidney already severely damaged by the subacute glomerulonephritis. However, after initial improvement the disease ran its progressive course.

The treatment of DIC should be directed towards preventing further coagulation by giving heparin in ordinary doses. Heparin must be given in spite of the bleeding tendency of the patient. In case 1 the DIC was interrupted by extirpation of the tumor after intensive replacement therapy, but it might have been safer to give her preoperative heparin treatment. However, failure with heparin treatment in DIC has also been reported (10), and the use of lytic substances such as urokinase has been suggested (13). The administration of lytic substances should be accompanied by continuous clotting studies of the patient.

SUMMARY

Disseminated intravascular coagulation (DIC) was a complication in 3 patients with different primary diseases. The probable initiation of the DIC was a thromboplastic active tumor, a laceration wound at cesarean section with amniotic embolism in the foetus and probable endotoxin release in a newly kidney biopsied patient with subacute glomerulonephritis. The DIC was interrupted in the first patient by extirpation of the tumor, in the other two by treatment with heparin in spite of bleeding tendency.

The diagnosis can be strongly suspected by demonstrating a decrease of the fibrinogen content combined with a diminished number of platelets. A normalization of the condition can

occur after interruption of the intravascular clotting by treatment with heparin

REFERENCES

1 Astrup T & Mullertz S The fibrin plate method for estimating fibrinolytic activity *Arch Biochem 40* 346 1952
2 Berglund G Immunoelectrophoretic studies of fibrinogen plasmin and lytic products of fibrinogen and fibrin *Int Arch Allergy 21* 193 1962
3 Bergstrom K Blomback B & Kleen G Studies on the plasma fibrinolytic activity *Acta Med Scand 168* 291 1960
4 Biggs R Eveling J & Richards G The assay of antihaemophilic globulin activity *Brit J Haemat 1 20 1955*
5 Blix S & Jacobsen C D The defibration syndrome in a patient with haemangio-endothelio sarcoma *Acta Med Scand 173* 377 1963
6 Bull B S Rubenberg M L Dacie J V & Brain M C Microangiopathic haemolytic anaemia mechanism of red cell fragmentation in vitro studies *Brit J Haemat 14* 643 1968
7 Corrigan J J Ray W L & May N Changes in the blood coagulation system associated with septicemia *New Eng J Med 279* 851 1968
8 Craig J M & Gitlin D The nature of the hyaline thrombi in thrombotic throbocytopenic purpura *Amer J Path 33* 251 1957
9 Deele J B A case of fatal haemorrhagic diathesis with premature detachment of the placenta *Amer J Obstet 44* 785 1901
10 Doughten R M & Pearson H A Disseminated intravascular coagulation associated with Aspergillus endocarditis *J Pediat 73* 576 1968
11 Hardaway R M *Syndromes of disseminated intravascular coagulation* Charles C Thomas Publ Springfield Ill USA 1966
12 Hathaway W E Mull M M & Pechet G S Disseminated intravascular coagulation in the newborn *Pediatrics 43* 233 1969
13 Lanzkowsky Ph & McCrory W Disseminated intravascular coagulations as a possible factor in the pathogenesis of thrombotic microangiopathy (hemolytic uremic syndrome) *J Pediat 70* 460 1967
14 McCluskey R T Benacerraf T B Potter J L & Miller F The pathologic affects of intravenously administered soluble antigen-antibody complexes I Passive serum sickness in mice *J Exp Med 111* 181 1960
15 McKay D G Gitlin D & Craig J M Immunochemical demonstration of fibrin in the generalized Shwartzman reaction *Arch Path 67* 270 1959
16 McKay D G Mansell H & Hertig A T Carcinoma of the body of the pancreas with fibrin thrombosis and fibrinogenopenia *Cancer 6* 682 1953
17 Merskey C Diagnosis and treatment of intravascular coagulation *Brit J Haemat 15* 523 1968
18 Noren I A specific two stage assay of prothrombin *Scand J Clin Lab Invest* To be published
19 Owren P A & Aas K The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin *Scand J Clin Lab Invest 3* 201 1951
20 Quick A J & Stefanini M The concentration of the labile factor of the prothrombin complex in human dog and rabbit blood its significance in the determination of prothrombin activity *J Lab Clin Med 33* 819 1948
21 Reid H A Chan K E & Thean P C Prolonged coagulation defect (defibrination syndrome) in Malayan viper bite *Lancet 1* 621 1963
22 Reid D E Weiner A E & Roby C C Intravascular coagulation and afibrinogenemia the presumptive lethal factors in the syndrome of amniotic fluid embolism *Amer J Obstet Gynec 66* 465 1953
23 Verstraete M & Vermylen J Acute and chronic defibrination in obstetrical practice *Thrombos Diathes Haemorrh 20* 444 1968
24 Leading article Microangiopathic haemolytic anaemia *Scott Med J 14* 187 1969

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A PATIENT WITH HEREDITARY GALACTOKINASE DEFICIENCY

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The normal metabolism of galactose takes place through a transformation to glucose which occurs in 3 steps each catalyzed by a separate enzyme The enzymes involved are

1 *Galactokinase* (ATP D-galactose 1 phosphotransferase E C 2 7 1 6)

2 *Galactose 1 phosphate uridyltransferase* (UDPGlucose α D galactose 1 phosphate uridylyltransferase E C 2 7 7 12) and

3 *UDPGalactose 4 epimerase* or galactowaldenase (UDPGlucose 4-epimerase E C 5 1 3 2)

No patient has yet been reported with deficiency of the third enzyme Deficiency of the second enzyme causes the classical form of hereditary galactosemia (11 19 20) clinically characterized by acute onset of jaundice and convulsions during the neonatal period later followed by evidence of liver cirrhosis mental retardation and cataracts In 1965 Gitzelman (13) reported the first case of hereditary galactokinase deficiency This case occurred in an adult who already in infancy had been recognized as having galactose diabetes (10) the patient had cataracts but showed no signs of liver involvement or of metal retardation The same metabolic defect has been demonstrated in one sister of this patient (14 15) another sister and a broth r have similar symptoms but have not been investigated biochemically Hereditary galactokinase deficiency has also been reported in a newborn infant in an Austrian family (31) unrelated to the previously described Swiss family both families are gypsies Mayes & Guthrie (22) during screening of 642 individuals from a mixed North American population found 6 persons in whom the galactokinase activity of the red blood cells was about half of the normal value these individuals were judged as heterozygotes for galactokinase deficiency To our knowledge no non Gypsy patients have however previously been reported with galactosemia due to galactokinase deficiency

CASE REPORT

The patient a boy born on June 24 1969 is the third child of healthy unrelated parents His brother and sister are healthy Pregnancy labour and delivery were normal birthweight was 3 760 g The boy was breast fed for the first few days and then received a formula (Milkotal® 140 g 5 times a day) containing 9–10 lactose He took 40–50 g eagerly but then appeared tired and the mother had some difficulties to make him take the whole meal each time His weight gain was a little slow and at age 3 weeks his weight was 90 g below the birth weight Other wise he was symptom free

At 3 weeks of age he was admitted to the Department of Paediatrics Jonkoping because a test for galactosemia (Guthrie's microbiological inhibition assay) performed in the neonatal period as a screening procedure was then reported abnormal He was a healthy looking normally developed infant Physical examination including a careful neurological examination revealed no abnormalities Particularly no liver enlargement could be demonstrated at any of the examinations The first ophthalmological examination which was performed when the boy was

The ophthalmological examinations were performed by Dr I Anjou

Table 1 *Galactokinase activity in hemolyzed red blood cells (radiochemical method (22 29) from the patient his relatives and a number of controls*

The controls have been divided into two groups according to age one infant group (2-4 months) with the higher galactokinase activity characteristic for the newborn period (25) and one group of adults and children (2-67 years) The values found in the controls agree well with earlier reports (22 25)

Subjects	Age	Galactokinase (units/ml erythrocytes)
Patient	3 mo	<0 015
Control P B	2 mo	1 360
Control M S	2 mo	0 483
Control T H	3 mo	1 275
Control R A	4 mo	0 460
Mother	24 y	0 097
Father	37 y	0 148
Brother	3 y	0 144
Sister	7 y	0 211
Control P A	2 y	0 372
Control P N	3 y	0 346
Control G S	6 y	0 319
Control C N	27 y	0 375
Control V L	28 y	0 394
Control A D	39 y	0 574
Control H P	67 y	0 305

5 weeks old and 10 days after dietary treatment had started revealed bilateral cataracts localized mainly in the posterior cortex of the lenses Routine laboratory examinations of blood and urine gave normal results except the test for reducing substance in the urine (clinitest) which was strongly positive Glucosuria tested with clinistix was absent on repeated examinations Blood sugar (4 hours after a meal) was 206 mg per 100 ml when determined as reducing substance and 63 mg per 100 ml when determined with glucose oxidase When galactose was assayed with galactose oxidase a concentration of 178 mg per 100 ml was found Serum bilirubin concentration transaminase and lactate dehydrogenase activities and thymol flocculation test were normal when examined both at 4 weeks and at 7 weeks of age Alkaline phosphatase was increased (45 U) at 4 weeks and within normal limits for age (22 U) at 7 weeks Serum protein including electrophoretic separation was normal as was the urinary excretion of amino acids An EEG recorded when the boy was 5 weeks old was judged as borderline because of the presence of some activity with low frequency and occasionally with questionable sharp wave form

When the boy was 3 / weeks old Milkotal® was replaced by a galactose free formula Sobee® His previously good general condition improved further in that he took this formula more eagerly than Milkotal® His weight gain during the last week on Milkotal® was 60 g and during the first week on Sobee® 300 g The galactose free diet has been continued and the boy s general condition has remained excellent he grows and develops normally The ophthalmological examination was repeated when he was 5 months old A marked improvement was then found as the opacities had diminished and now consisted only of a thin layer in the posterior cortex the eye ground could be clearly seen and was normal

SPECIAL INVESTIGATIONS

Galactose in urine The boy s urine gave a strongly positive reaction with a galactose specific test paper (6)

For the quantitative assay of galactose in urine we used galactose dehydrogenase (D galactose NAD oxidoreductase E C 1 1 1 48) (Boehringer und Soehne GmbH, Mannheim Germany) in the following way to 100 μl of urine (no pre treatment necessary) was added 1 000 μl of 100 mM TRIS-HCl buffer pH 8 6 and 50 μl of NAD solution (10 mg per ml) After a stable reading at 340 mμ had been obtained 5 μl of galactose dehydrogenase (crystal suspension 5 mg per ml) was added, and the contents of the cuvette was well mixed by stirring with a plastic rod Reading was then made intermittently until a new plateau had been reached (9 27 33) The amount of galactose was calculated from the increase in optical density If the increase was larger than 1 000 a new assay was performed with a diluted urine sample The galactose concentration, determined with this method when the boy was 3 weeks old was found to be 2 550 mg per 100 ml urine

Earlier urine samples were not available for analysis, since galactose test paper screening of urine is not performed in the hospital where he was born and his mild early symptoms caused no concern and gave no clue to a diagnosis After the introduction of galactose free diet the galactose concentration rapidly decreased and after 1 week it was less than 4 mg per 100 ml urine

If galactose in urine is assayed with galactose oxidase interfering substances first must be removed by ion exchange filtration (30)

Galactitol in urine Galactitol in urine was assayed in the same way as in a previous investigation (8) In the sample obtained before introduction of galactose free diet there was 340 mg per 100 ml urine and in the sample obtained 1 week later 104 mg per 100 ml urine Thus when the patient is on a galactose free diet the urinary excretion of galactitol seems to persist longer than that of galactose

Galactose 1 phosphate uridyltransferase activity in red blood cells As a preliminary test for the demonstration of galactose 1 phosphate uridyltransferase activity we used the fluorescent spot test of Beutler & Baluda (2) A normal reaction was obtained indicating the presence of galactose 1 phosphate uridyltransferase For the quantitative assay of galactose-1 phosphate uridyltranferase activity we used the method of Tolstrup (3 32) which is based on UDPG consumption in the presence of galactose 1 phosphate (1) The hemolysate was pre-incubated with dithiothreitol which activates the enzyme (8 24) The transferase activity was calculated as units (μmoles of UDPG consumed per hour) per g hemoglobin The activity found was 35 6 units per g hemoglobin which is a normal activity (it is even in the upper normal range since our normal value is 26 9 ± 4 5 (S D) units per g hemoglobin) In patients with the classical form of hereditary galactosemia this enzyme is absent

Galactokinase activity in red blood cells The galactokinase activity in red blood cells was measured with the method of Mayes & Guthrie (22) which is a modification of that originally described by Sherman (29) Heparinized venous blood was used

The blood samples were stored at 0–4 C for a maximum of 24 hours before analysis due to the limited stability of the enzyme (22) Control blood samples were treated in exactly the same way as those from the patient and his relatives The red blood cells were washed with cold saline and then hemolyzed by freezing and thawing just before the incubation The amount of radioactive galactose phosphorylated during the reaction and consequently adsorbed onto

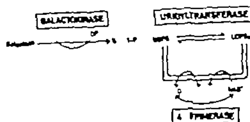


Fig 1 In the normal subject exogeneous galactose is converted into glucose by a reaction sequence catalyzed by three separate enzymes The deficiency of either one of these enzymes will lead to a state of galactosemia in each case with its own biochemical and clinical characteristics

ion exchange paper was measured in a Packard liquid scintillation counter (35) The galactokinase activity was calculated as units (μmoles of galactose phosporylated per hour) per ml packed red blood cells The activity found in the samples from the patient was essentially zero when compared with the activity found in the controls (Table 1) A very weak residual activity less than a few per cent of the normal could be detected This has been seen also in the other patients with galactokinase deficiency (Gitzelmann personal communication) It seems more likely that this residual activity is caused by a weak unspecific effect of some other enzyme than by a small residue of the enzyme in which the patient is deficient

Normally the galactokinase activity is higher in early infancy than later in life (25) and therefore controls of different ages have been included in Table 1 It is seen that both the mother and the father of the patient have values for galactokinase activity which are only about the half of the normal which clearly fits with the concept that both parents are heterozygotes for the disease Also the brother appears to be a heterozygote whereas the sister has an intermediate value which does not permit any sure conclusion as to whether she is a heterozygote or not

Galactose 1 phosphate in erythrocytes In the classical form of hereditary galactosemia caused by galactose 1 phosphate uridyltransferase deficiency galactose 1 phosphate is accumulated in the erythrocytes on galactose in

take In patients with hereditary galactokinase deficiency this should not be expected to occur since the enzyme catalyzing the formation of galactose 1-phosphate is missing In accord with this we did not find any galactose 1-phosphate when we analysed with a fluorimetric method (7) washed erythrocytes obtained shortly after the introduction of galactose free diet Neither was any galactose 1-phosphate found after that the erythrocytes had been pre incubated with galactose (150 mg per 100 ml) at 37 C for 30 min

DISCUSSION

Theoretically a lack of either one of 3 different enzymes could produce galactosemia, each en zyme deficiency causing its own clinical and biochemical abnormalities (Fig 1) The lack of UDPGalactose 4 epimerase has so far not been demonstrated Normal epimerase activity has been found in patients with galactose 1-phos phate uridyltransferase deficiency (21) and in patients with galactokinase deficiency (14 31), it was not examined in our patient There is no reason to suppose a genetic coupling between the epimerase and the other 2 enzymes If epimerase deficiency occurs, it would be ex pected to occur as an isolated defect

There is however reason to believe that a fetus with epimerase deficiency may not be viable Epimerase transforms UDPGlucose into UDPGalactose, which is the immediate precursor for the galactose part of the complex galactose-containing molecules necessary for the building of the central nervous system This enzyme is thus involved in a vital process The two other enzymes do not participate in this endogenous galactose formation

A lack of galactose-1 phosphate uridyltrans ferase causes the classical form of hereditary galactosemia This disease has an acute onset with the appearance of serious symptoms vom iting weight loss, jaundice and convulsions within a few days after milk feeding has started Large amounts of galactose and galactitol are excreted in the urine and marked aminoacid uria, due to liver and kidney damage (8), is common If the patient survives but still re ceives galactose-containing food he will develop cataracts, mental retardation and liver cirrhosis

The syndrome caused by galactokinase defi ciency is milder In spite of an urinary excretion of galactose and galactitol which is of the same magnitude as in galactose-1 phosphate uridyl transferase deficiency acute clinical symptoms are absent Reported adult patients have shown cataracts but neither mental retardation nor liver involvement The lack of early clinical symptoms was predicted by Gitzelman (14) after his study of the first adult patients and was confirmed first by Thalhammer Gitzel mann & Pantlitschko (31) and now by us

The difference between the two syndromes concerning the presence of acute clinical symp toms may have a biochemical explanation In galactose 1 phosphate uridyltranferase defici ency the administration of dietary galactose or lactose leads to the accumulation of galactose 1 phosphate in the tissues, while in galacto-kinase deficiency galactose 1 phosphate can not be formed from exogeneous galactose It therefore seems to be the sugar phosphate rather than free galactose or the sugar alcohol which causes the toxic symptoms from liver kidneys and central nervous system This has an interesting parallel in two disturbances of the metabolism of fructose In *fructose intolerance* (4) caused by the deficiency of liver aldolase (5 12 19) the administration of fructose leads to immediate clinical symptoms (vomiting tre mor convulsions) In this disease fructose can be phosphorylated but then not further meta bolized In *essential fructosuria* (first described by Zimmer (36) there is a deficiency of anoth er enzyme necessary for the metabolism of exogeneous fructose namely fructokinase (5 19 23 28) In the later defect fructose cannot be phosphorylated Although the administra tion of fructose leads to at least equally marked excretion of fructose with the urine as in fruc tose intolerance, there are no clinical symptoms whatsoever Apparently the tissue accumulation to unmetabolized hexose phosphates whether

galactose or fructose has a pronounced disturbing effect while a high concentration of hexose as such has milder if any metabolic effects

The one important clinical defect caused by galactokinase deficiency the cataracts also has a biochemical explanation Galactitol can be isolated from the lens and other tissues of rats which have been fed high galactose diet leading to cataracts (18 26) and it is likely that deposition *of the sugar alcohol is responsible for the* opacity of the lens Both in galactose 1 phosphate uridyltransferase deficiency (8 34) and in galactokinase deficiency (13 14—also verified in the present case) considerable amounts of galactitol are excreted in the urine The formation of galactitol from galactose is catalyzed by the enzyme aldose reductase which does not require previous phosphorylation of the substrate and thus offers an alternative pathway when the ordinary metabolism of galactose is blocked The tissue distribution of aldose reductase is not known (14 17) but it is known to be present in the lens (16)

Although the metabolism of administered galactose mainly occurs in the liver the diagnosis of galactokinase deficiency as well as that of galactose 1 phosphate uridyltransferase deficiency is based on the assay of enzymes in the erythrocytes In both cases the enzyme in red blood cells appears to be controlled by the same gene as the enzyme in the liver Like the galactose 1 phosphate uridyltransferase deficiency the galactokinase deficiency is recessively inherited, and the heterozygotes can be identified on the finding of enzyme activity reduced to roughly half of the normal value (15) Both parents of our patient had a reduced enzyme activity a finding to be expected as both of them must be heterozygotes Besides the patient s brother and possibly also his sister appear to be heterozygotes

The incidence of reduced enzyme activity was found to be about 1 in 100 when examined in an unselected group of American individuals (22) Provided the same frequency is found in a larger population the incidence of galactosemia due to galactokinase deficiency can be estimated to roughly 1 in 40 000 new born infants

The most serious sequela of the disorder apparently is the cataract Galactokinase deficiency should be kept in mind as a possible cause of cataracts in infancy and childhood when no other explanation is apparent In our case opacities of the lenses were already present in the 5 weeks old infant who had received galactose containing food for altogether $3\,^1/_2$ weeks A considerable improvement was noted 4 months later during this time the infant received only galactose free food It it thus desirable to detect the metabolic defect and start dietary treatment as early as possible and long before the cataracts have given obvious clinical symptoms As no symptoms precede the cataract the attainment of this goal means the introduction of a screening procedure of all new borns All screening methods for hereditary galactosemia which are based on the demonstration of increased blood or urinary galactose concentration will detect also galactokinase deficiency as in fact happened in our patient The increased blood galactose concentration persists only for a limited period after a meal since there is a rapid urinary excretion plus some metabolism catalyzed by galactose dehydrogenase in the liver and possibly also by aldose reductase (31) a source of error which must not be neglected when blood galactose is used as the screening test for the various forms of galactosemia This factor may explain why in a screening of 327 000 newborn babies no case of galactokinase deficiency was found (Guthrie 1966 cited by Thalhammer et al (31) Urinary screening may be less dependent on the interval between feeding and sampling but has the disadvantage that in a newborn a urine sample is more difficult to obtain than a blood sample

In screening for galactose 1 phosphate uridyltransferase deficiency a simple fluorescence spot test for the enzyme activity in blood cells has been described (2) This test has the advantage that it is independent of dietary galactose (lactose) administration and thus can be performed on cord blood immediately after

birth Since this test is specific for galactose 1 phosphate uridyltranferase it will however, not detect galactokinase deficiency and our patient also showed a normal spot test reaction If screening for defects in galactose metabolism is based on this kind of tests, therefore a separate test will have to be performed for galactokinase We made attempts to perfom a spot test for galactokinase deficiency by replacing the galactose-1 phosphate, used as substrate in the uridyltransferase spot test with galactose plus ATP but these attempts were not fruitful since an unspecific fluorescence developed in the presence of ATP also if galactose was excluded This fluorescence is most probably due to the formation and oxidation of glucose-6 phosphate when ATP is added

SUMMARY

A case of hereditary galactokinase deficiency is reported occurring in a newborn boy and detected through routine screening of newborns for increased blood concentration of galactose Symptoms were equivocal and consisted only of mild feeding difficulties and a slightly insufficient weight gain The only abnormality found on clinical examination was lens opacities which were obvious when the boy was 5 weeks old The biochemical abnormalities found were excretion of large amounts of galactose and galactitol in the urine, galactosemia, and essentially no galactokinase activity in the red blood cells On a galactose free diet, introduced when the boy was 3 1/ weeks old he grew and developed normally his cataracts had improved considerably after 4 months The boy's both parents one brother and possibly one sister were considered carriers of the autosomal recessive gene, as the enzyme activity found in their red blood cells was roughly half of the normal value

The clinical findings in patients with the various enzyme defects leading to galactosemia are described and related to the biochemical abnormalities caused by the defects Possible screening methods are discussed

ACKNOWLEDGMENT

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REFERENCES

1 Anderson E P Kalckar H M Kurahashi K & Isselbacher K J A specific enzymatic assay for the diagnosis of congenital galactosemia *J Lab Clin Med 50* 469 1957

2 Beutler E & Baluda M C A simple spot screening test for galactosemia *J Lab Clin Med 68 137 1966*

3 Brandt N J *Galaktose 1 fosfat uridyl transferase Hereditær galaktosæmi leverceirrhose og cirrhose* Dissertation Munksgaard Forlag Copenhagen 1966

4 Chambers R A & Pratt R T C Idiosyncrasy to fructose *Lancet II 340 1956*

5 Cornblath M & Schwartz R *Disorders of carbohydrate metabolism in infancy* Saunders, Philadelphia and London 1966

6 Dahlqvist A Test paper for galactose in urine *Scand J Clin Lab Invest 22* 87 1968

7 — To be published 1970

8 Dahlqvist A Jagenburg R & Mark A A patient with hereditary galactosemia studied with a screening method for galactose in urine *Acta Pædiat Scand 58 237 1969*

9 Doudoroff M D Galactose dehydrogenase of Pseudomonas saccharophila *in* S P Colowick & N O Kaplan (eds) *Methods in enzymology* vol V Academic Press New York and London 1962

10 Fanconi G Hochgradige Galaktose Intoleranz (Galaktose Diabetes) bei einem Kinde mit Neurofibromatosis Recklinghausen *Jb Kinderheilk 138* 1 1933

11 — Die angeborene Störungen des Kohlenhydratstoffwechsels *in* H Opitz & F Schmid (eds) *Handbuch der Kinderheilkunde* vol IV Stoffwechsel Ernährung und Verdauung Springer Verlag Berlin Heidelberg and New York 1965

12 Froesch E R Wolf H P Baitsch H Prader A & Labhart A Hereditary fructose intolerance An inborn defect of hepatic fructose 1 phosphate splitting adolase *Amer J Med 34* 151 1963

13 Gitzelmann R Deficiency of erythrocyte galactokinase in a patient with galactose diabetes *Lancet II* 670 1965

14 — Hereditary galactokinase deficiency a newly recognized cause of juvenile cataracts *Pediat Res 1* 14 1967

15 — Galactokinase deficiency *in* D Y Y Hsia (ed) *Galactosemia* C C Thomas Publisher Springfield 1969

16 Hayman S & Kinoshita J H Isolation and

properties of lens aldolase reductase *J Biol Chem* *240* 877 1965

17 Hers H G L-aldose reductase *Biochim Biophys Acta* *37* 120 1960

18 van Heyningen R Formation of polyols by the lens of the rat with sugar cataract *Nature* *184* 194 1959

19 Hsia D Y Y *Inborn errors of metabolism* part 1 Clinical aspects Year Book Medical Publishers Chicago 1966

20 Isselbacher K J Anderson E P Kurahashi K & Kalckar H M Congenital galactosemia a single enzymatic block in galactose metabolism *Science* *123* 635 1956

21 Kalckar H M Anderson E P & Isselbacher K J Galactosemia a congenital defect in a nucleotide transferase *Biochim Biophys Acta* *20* 262 1956

22 Mayes J S & Guthrie R Detection of heterozygotes for galactokinase deficiency in a human population *Biochem Genet* 2 219 1968

23 Marks V & Samols E Glucosurias other than diabetes mellitus in F Dickens P J Randle & W J Whelan (eds) *Carbohydrate metabolism and its disorders* vol 2 Academic Press London and New York 1968

24 Mellman W J & Tedesco T A An improved assay of erythrocyte and leukocyte galactose 1 phosphate uridyl transferase stabilization of the enzyme by a thiol protective reagent *J Lab Clin Med* 66 940 1965

25 NG W G Donnell G N & Bergren W R Galactokinase activity in human erythrocytes of individuals of different ages *J Lab Clin Med* *66* 115 1965

26 Quan Ma R & Wells W W The distribution of galactitol in tissues of rats fed galactose *Biochem Biophys Res Commun* *20* 486 1965

27 Reinhardt H & Hollmann S Bestimmungsmethoden von D-Glucose und niedermolekularen Kohlenhydraten III D-Galactose in H Bartelheimer W Heyde & W Thorn (eds) *D-Glukose und verwandte Verbindungen in Medizin und Biologie* Ferdinand Enke Verlag Stuttgart 1966

28 Schapira F Schapira G & Dreyfus J C La lésion enzymatique de la fructosurie bénigne *Enzym Biol Clin (Basel)* *1* 170 1962

29 Sherman J R Rapid enzyme assay technique utilizing radioactive substrate ion-exchange paper and liquid scintillation counting *Anal Biochem* *5* 548 1963

30 Tengstrom B Enzymatic determination of glucose and galactose in urine *Scand J Clin Lab Invest* *18* Suppl 92 104 1966

31 Thalhammer O Gitzelmann R & Pantlitschko M Hypergalactosemia and galactosuria due to galactokinase deficiency in a newborn *Pediatrics* *42* 441 1968

32 Tolstrup N *Hereditær galaktosæmi Galaktose 1 fosfaturidyltransferase målinger med henblik på diagnose og arvegang* Dissertation Arne Frost Hansens Forlag Copenhagen 1965

33 Wallenfels K & Kurz G Über die Spezifität der Galaktosedehydrogenase und deren Anwendung als analytisches Hilfsmittel *Biochem Z* *335* 559 1962

34 Wells W W Pittman T A & Egan T J The isolation and identification of galactitol from the urine of patients with galactosemia *J Biol Chem* 239 3192 1964

35 Wilson D B & Hogness D S Galactokinase and uridine diphosphogalactose 4-epimerase from Escherichia coli in S P Colowick & N O Kaplan (eds) *Methods in enzymology* vol VIII Academic Press New York and London 1966

36 Zimmer K Levulose im Harn eines Diabetikers *Deutsch Med Wschr* *2* 329 1876

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C REACTIVE PROTEIN IN SERUM FROM INFANTS AS DETERMINED WITH IMMUNODIFFUSION TECHNIQUES

II *Infants with Various Infections*

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Several authors have shown that infants are able to form C reactive protein (CRP) during the course of various infectious diseases (1 a 1 5) In these investigations employing the capillary tube precipitation method, the diagnostic usefulness of CRP in small infants has been suggested

With the more accurate and sensitive immunodiffusion methods CRP has been detected in 17% of infants apparently healthy on clinical examination (6) Usually it was present only in trace amounts however The same techniques have now been applied in a study of the occurrence of CRP in infants hospitalized because of various infections

MATERIAL AND METHODS

Serum samples were obtained from 113 infants 1–15 months of age admitted to the hospital because of an infectious disease The samples were obtained within 10 days of the onset of the disease in these patients Nineteen were diagnosed as having acute pyelonephritis caused by Gram negative bacteria From a few of the patients with recurrent attacks of pyelonephritis consecutive blood samples were obtained Thirty were diagnosed as gastroenteritis 33 as bronchitis or bronchopneumonia and 23 as upper respiratory infection or otitis In the latter group the presence or absence of bacterial pathogens in throat cultures was recorded Finally 8 patients were included with evidence of viral infections (2 with serous meningitis 4 with exanthema subitum and 2 with ECHO infections) The sera were tested within 24 hours of sampling

The CRP determinations were performed with the comparative double diffusion in gel technique and the single radial immunodiffusion method as earlier described (4) Concentrations of 0 5–1 0 μg/ml of CRP are recorded as trace amounts and higher concentrations are expressed in μg/ml

RESULTS

No significant relationship was seen between the age of the infants and the CRP concentrations found (Fig 1 *a*) On the other hand a correlation was observed between the CRP concentrations and the sedimentation rates of the blood samples (Fig 1 *b*)

CRP was found in all sera from infants with pyelonephritis often in high amounts (Fig 2 *a* and Table 1) This was seen both in patients with an acute onset of disease and in those where an infection was discovered by positive urinary cultures on a routine check up CRP was studied in consecutive serum samples from some patients A rapid rise to a high CRP concentration after onset of infection was seen as exemplified by patient H M in Fig 3 After treatment of the infection the CRP concentration decreased quickly below demonstrable quantities as shown by patient A E in the same figure This patient again had a ru

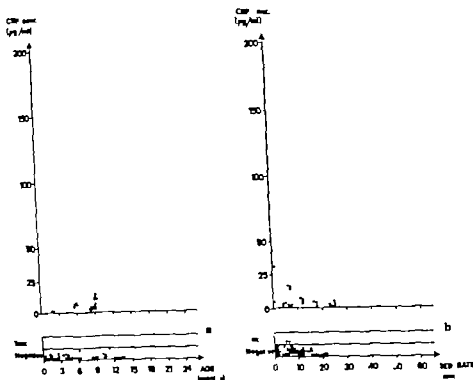


Fig 1 (a) Relation between CRP concentration and age in 113 infected infants (b) Relation between CRP concentration and sedimentation rate in 113 infants

nor temporary rise in CRP concentration without any clinical or laboratory signs of recurrent infection But at 14 weeks a symptomatic recurrent attack of pyelonephritis was accompanied by high CRP concentrations

In the infants with gastroenteritis CRP could not be detected in 19 of the 30 patients where as it was present in trace amounts in two and in rather low concentrations in the remaining nine (Fig 2 *b*)

In the 33 infants with bronchitis or bronchopneumonia, CRP was demonstrable in all but 7 patients (Fig 2 *c* and Table 1)

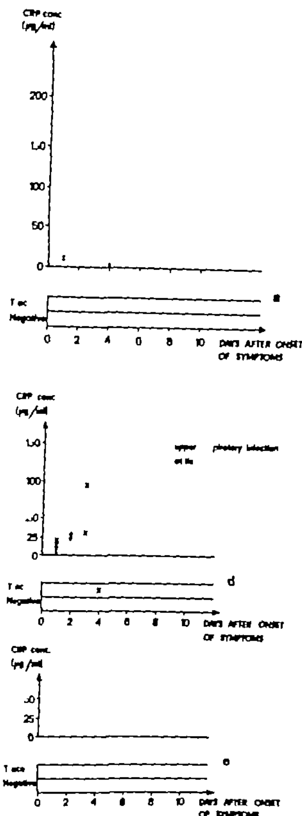
CRP was found in all of the 7 patients with otitis and 12 of the 16 patients with acute pharyngitis (Fig 2 *d* and Table 1) In the combined otitis–pharyngitis group 12 patients had pathogenic bacteria (*Hemoph influenza pneumococci staphylococci β hemolytic streptococci* and *coliform organisms*) cultured from their throats These 12 patients had CRP in their sera In contrast the 4 patients without CRP had ordinary throat cultures (Table 2)

Finally in the small group of 8 patients with evidence of a viral infection CRP was detected in five instances (Fig 2 *e* and Table 1)

DISCUSSION

It has been suggested that CRP determinations could be of diagnostic help especially for infectious diseases in early infancy (1 6) Using the capillary tube precipitation technique Felix et al (1) found that CRP appeared in sera from two-thirds of infected infants in contrast to their normal controls where CRP was found in serum in a frequency of 27%

In this study quantitative determinations of CRP were performed with immunodiffusion methods which are known to be more sensitive specific and accurate than the generally employed capillary tube precipitation method



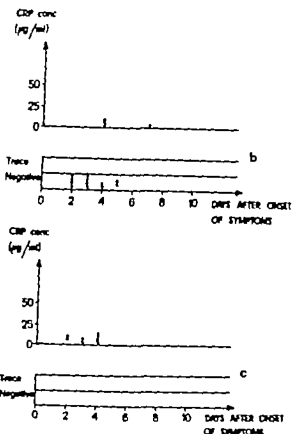


Fig 2 Serum content of CRP in (*a*) 19 infants with pyelonephritis (*b*) 30 with gastroenteritis (*c*) 33 with bronchitis or bronchopneumonia (*d*) 23 with pharyngitis or otitis and (*e*) 8 with viral infections The lower part of each figure shows those samples which had only traces of CRP or were negative on analysis with the double diffusion method Those positive with this method were quantitated by the single radial diffusion technique as is depicted in the diagram above

(4) Using these techniques CRP was demonstrated in amounts of > 1 μg/ml in sera from about two thirds of infected infants With the same methods CRP was found in concentrations exceeding 1 μg/ml in only 4% of apparently healthy infants (6) During the course of infections such as gastroenteritis it was observed in only one third of the cases whereas it was seen in all cases of pyelonephritis The reason for such variations may be differences in the severity and localization of the infections as well as in causative microorganisms It is sug

Table 1 *CRP in serum in relation to various infectious diseases*

Diagnosis	No of patients	C reactive protein (CRP) Present	Trace	Not demonstrable
Pyelonephritis	19	18	1	0
Gastroenteritis	30	9	2	19
Bronchitis or broncho-pneumonia	33	24	2	7
Otitis or pharyngitis	23	17	2	4
Viral infections	8			
serous meningitis	2	0	0	2
exanthema subitum	4	2	1	1
ECHO infection	2	2	0	0
Total	113	72	8	33

gestive that all the patients with pyelonephritis caused by Gram negative bacteria as well as those with upper respiratory infections with bacterial pathogens isolated in throat cultures had demonstrable CRP In the small group of 8 patients with presumed *viral infections* CRP was not detectable in three This as well as the low frequency of CRP appearing among the patients with gastroenteritis presumably mostly *of viral etiology* may support the earlier observation of several authors that bacterial infections more often induce the formation of CRP than do viral infections (3)

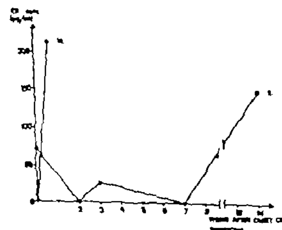


Fig 5 CRP content in consecutive serum samples from patients with pyelonephritis

Table 2 *CRP in serum related to throat culture findings in children with otitis or pharyngitis*

	CRP demonstrable	CRP not demonstrable
Bacterial pathogens in throat cultures	12	0
Ordinary throat culture	7	4

Studies of consecutive samples from a few patients with recurrent attacks of pyelonephritis indicated that the CRP concentration rapidly increased to high values after the onset of infection and decreased after adequate treatment This indicates that CRP may be a good index of the course of pyelonephritic infection

These findings suggest that in infants with *certain types of infections especially bacterial* infections quantitative CRP determination can *be of help for diagnosis and follow up* It seems worthwhile to evaluate further the relation of CRP to the course of pyelonephritis

SUMMARY

Quantitative CRP determinations using immunodiffusion methods have been performed on 113 infants with various infections Occurrence and concentration of CRP differed for various infectious diseases Thus it was found in only a third of the infants with gastroenteritis whereas it was found in all infants with pyelonephritis mostly in high concentrations

REFERENCES

1 Felix N S Nakajima H & Kagan B Serum C reactive protein in infections during the first six months of life *Pediatrics 37* 270 1966
2 Hanson L A & Nilsson L A Studies in C reactive protein during the pre and neonatal period *Acta Path Microbiol Scand 56* 409 1962
3 Hedlund P Clinical and experimental studies on C reactive protein (acute phase protein) *Acta Med Scand Suppl 361* 1961 p 71
4 Nilsson L A Comparative testing of precipitation methods for quantitation of C reactive protein in blood serum *Acta Path Microbiol Scand* 73 129 1968

5 Philipson L & Tveterås E C reactive protein in infancy Its appearance during the first year of life transplacental passage and electrophoretic pattern *Acta Paediat Scand 46* 1 1957
6 Saxstad J Nilsson L Å & Hanson L Å C reactive protein in serum from infants as determined with immunodiffusion techniques I Healthy infants *Acta Paediat Scand 59* 25 1970

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A DOUBLE BLIND STUDY OF THE EFFECT OF PHENOBARBITONE ON NEONATAL HYPERBILIRUBINAEMIA AND FREQUENCY OF EXCHANGE TRANSFUSION

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It has been observed that infants of mothers treated with phenobarbitone because of epilepsy or preeclampsia had a lower incidence of serum bilirubin levels above 10 mg/100 ml than controls A less clearcut effect was seen when phenobarbitone injections were given to the infants after birth (9) In a more detailed study the administration to pregnant women, the infants only or to both mothers and infants was compared (10) Best results were obtained again when the mother was treated for at least a fortnight before delivery but treating the infants by a total of 10 injections gave closely comparable results This effect of phenobarbitone therapy in neonatal jaundice has been confirmed (5 6) The treatment of the infant alone however was less effective than treating the mother (5) Yeung & Field (13) in Hongkong not only saw an effect on serum bilirubin but also on the frequency of exchange transfusions needed because of high serum bilirubin When treatment was started after neonatal jaundice had appeared phenobarbitone did not seem to have an effect (3)

In premature infants few studies seem to have been done In a retrospective analysis no significant difference between infants of mothers who had or had not received barbiturates during pregnancy could be demonstrated (12) With Nikethamid orally no difference in the frequency of exchange transfusion was found although serum bilirubin was lower in the treated group on the 6th day (4)

Because the premature infants are those most likely to develop dangerous hyperbilirubinaemia which necessitates exchange transfusion, it seemed indicated to study the influence of phenobarbitone on serum bilirubin concentration and frequency of exchange transfusion especially in this age group

PATIENTS AND METHODS

Newborns admitted to the children's hospital within 24 hours after delivery because of prematurity respiratory difficulties or other reasons were randomly assigned to either the treated or the control group Neither to the physician nor the nurses was it known whether an infant was receiving placebo or phenobarbitone

A total of 10 injections of phenobarbitone or placebo solution was given spaced 8 hours apart. The first injection was given as soon as possible after admittance The single dose injected was the following for newborns less than 1 000 g body weight 0 1 ml (1 mg) between 1 000 and 1 500 g 0 2 ml (2 mg) between 1 500 and 2 000 g 0 3 ml (3 mg) and so on to a maximum of 0.5 ml (5 mg) for infants weighing 2 501 g or more The total dose per 24 hours varied therefore between 3 mg and 15 mg of phenobarbitone

Serum bilirubin concentration was measured daily for 10 days and less frequently red cells haemoglobin haematocrit reticulocytes prothrombin time urea and blood pH was estimated Every child was carefully observed for side effects by one of us (E. S)

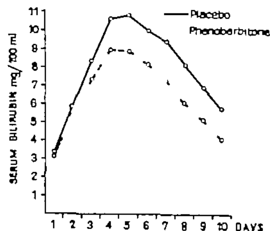


Fig 1 Serumbilirubin levels in control and phenobarbitone treated infants (Mean values of all infants)

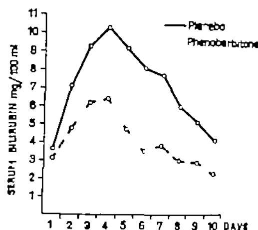


Fig 3 Serumbilirubin levels in control and phenobarbitone treated full term infants

140 infants were assigned to the study 70 in each group Cases with Rh or ABO incompatibility and critically ill infants were excluded from the study

RESULTS

From the 140 infants 10 in the treated and 8 in the control group died with one exception within 3 days after birth The difference in deads in the two groups is not significant by the χ test Four infants dropped from the treated and 2 from the control group for various reasons Thus there were 56 newborns in the barbiturate and 60 in the placebo group Of these 43 respectively 46 had a body weight of less than 2 500 g In the whole group the mean weight was 2 070 g in the treated and 2 203 g in the control group The mean weight of the infants with less than 2 500 g body weight was 1 805 g (treated) and 1 872 g (controls) Mean gestational age was 44 days before term in the former and 47 5 days in the latter group

Frequency of exchange transfusions There were 11 exchange transfusions in the placebo and 1 in the treated group This difference by the χ^2 test is significant ($p < 0.01$) All exchange transfusions with one exception had to be performed in infants of less than 2 500 g

Serumbilirubin concentration The course of serumbilirubin of the two groups is shown in Fig 1 The placebo group has significantly higher values from the fourth day on (In the t test $p < 0.025$ on day 7 to 9 $p < 0.01$) If one regards premature and full term infants separately the difference in bilirubin concentration in the treated and control group is more prominent in the latter In the premature group this difference is significant at a 5° level on the 7th and at a 1% level between the 8th and 10th day (Fig 2) In the small group of fullterm infants (13 and 14 infants) p is lower than 0 05 on the third and fourth day and lower than 0 025 on the 5th and 6th day (Fig 3)

In contrast to the difference in bilirubin levels red cell count haemoglobin reticulocytes haematocrit prothrombin urea and blood pH were identical in both groups

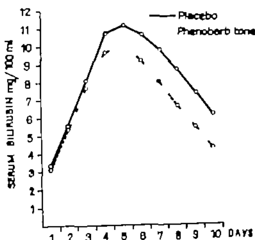


Fig 2 Serumbilirubin levels in control and phenobarbitone treated premature infants

DISCUSSION

The present investigation indicates that in premature infants the number of exchange transfusions can be decreased significantly by treatment with small doses of phenobarbitone after delivery. This confirms the results of Yeung & Field (13). The doses used were always lower than the amount of 6 to 7 mg/per kg/24 h given to newborn infants as a sedative and far below that given in convulsions.

The average serumbilirubin levels are also lower in the treated infants. The decrease is more pronounced in fullterm than in premature infants. This has also been observed by Ramboer et al. (6). The rather low dose used in the lowest weight classes (only 3 mg/24 h in infants less than 1 000 g) might contribute to this result.

When one considers the definite mortality of exchange transfusion, especially in low weight prematures with other disturbances, e.g. respiratory distress syndrome, phenobarbitone treatment seems to offer an alternative. Against this a possible danger of the drug therapy has to be carefully balanced.

As has been mentioned, a daily detailed clinical examination was performed without knowing whether an infant belonged to the placebo or treated group. The results were recorded. Evaluation of these notes showed that there was no discernible effect of phenobarbitone on general behaviour, motor activity, feeding, respiration, reflexes etc. This was also the experience of Trolle (10). The measurement of blood pH, P_{CO_2} and standard bicarbonate showed no ill effects. In view of the fact, that phenobarbitone has been used for decades in newborn infants for a variety of reasons and in higher dosage, we feel that untoward effects of the small doses used during only 3 days are unlikely.

Trolle (10) has investigated whether the albumin binding of unconjugated bilirubin is decreased by the phenobarbitone. No competing was found. It is therefore improbable that the observed decrease of serum bilirubin is related to an alteration of protein binding. In addition Valaes & Doxiadis (11) demonstrated that the faecal excretion of bile pigment is increased by phenobarbitone.

It has been shown that phenobarbitone and other substances induce the synthesis of hepatic microsomal enzymes including glucuronyl transferase (2, 7). An enhancement not only of bilirubin conjugation but also of biliary excretion by phenobarbitone has been shown in animals (1, 8). A similar mechanism might also cause the lower concentration and quicker decrease of serum bilirubin in infants.

SUMMARY

In a double blind study the effect of phenobarbitone on neonatal hyperbilirubinaemia and the frequency of exchange transfusion was investigated. Newborn infants admitted were randomly assigned to one of two groups. The treated group comprised 56 and the control group 60 infants. Either placebo solution or phenobarbitone was injected i.m. at 8 hourly intervals for a total of 10 injections. The single dose varied between 1 mg and 5 mg according to weight. One exchange transfusion had to be performed in the treated and 11 in the control group. This difference is statistically significant. Serumbilirubin concentrations from the 4th day on were significantly lower in the treated infants than in controls.

REFERENCES

1. Catz, C. & Yaffe, S. J.: Barbiturate enhancement of bilirubin conjugation and excretion in young and adult animals. *Pediat Res 2*: 361, 1965.
2. Conney, A. H.: Pharmacological implications of microsomal enzyme induction. *Pharmacol Rev 19*: 317, 1969.
3. Cunningham, M. D., Mace, J. W. & Peters, E. R.: Clinical experience with phenobarbitone in icterus neonatorum. *Lancet 1*: 550, 1969.
4. Kintzel, H. W. & Hinkel, G. K.: Der Einfluss von Nikethamid auf den Serumbilirubinspiegel des Frühgeborenen. *Mschr Kinderheilk 117*: 84, 1969.
5. Maurer, A. M., Wolff, J. A., Finster, M., Poppers, P. J., Pantuck, E., Kuntzman, R. & Conney, A. H.: Reduction in concentration of total

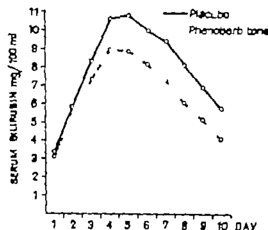


Fig 1 Serumbilirubin levels in control and phenobarbitone treated infants (Mean values of all infants)

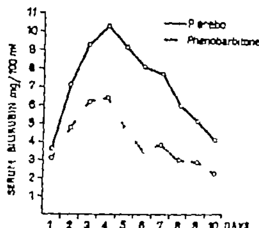


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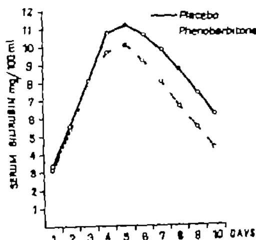


Fig 2 Serumbilirubin levels in control and phenobarbitone treated premature infants

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SUDDEN UNEXPECTED DEATH IN INFANCY

An Autopsy Study

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The etiology and pathogenesis of sudden and unexpected death in apparently healthy infants (SUD) is still an unsolved problem A variety of pathogenetic mechanisms have been suggested (7 8 16) among which are status thymico-lymphaticus accidental suffocation by bedclothes anaphylactic reaction to cow's milk (12 13) and hyperacute bacterial or viral infections All of the proposed hypotheses have weak points either from evidence that is in direct opposition to the hypothesis or from the lack of supportive evidence The thymus is not larger in SUD than in other cases of sudden death (9) the composition of the inspired air is not significantly altered under the cover of ordinary bedclothes (17) serum protein studies in SUD victims have given no evidence for the presence of allergy to cow's milk (10 13 14 15) microbiological post mortem examinations have usually failed to show pathogenic organisms in the majority of cases (2 6 15)

The SUD cases autopsied at Ullevål hospital over a 10-year period have been reviewed For comparison sudden deaths from non infectious causes and deaths from acute infections in the same age group have been included The aim has been to fit the autopsy findings in SUD into a spectrum of findings in cases of explained deaths thus seeking evidence for the nature of SUD

MATERIAL AND METHODS

During the 10-year period 1959–1968 207 infants between 2 weeks and [illegible] years of age were autopsied at Ullevål hospital From anamnestic data, 53 of these cases were selected for the present study The material was divided into five diagnostic groups for which abbreviated designations will be used

SUD sudden unexpected death 17 boys and 9 girls The group comprises infants in whom no sign of illness except trivial symptoms of common cold had been noted prior to death and in whom autopsy findings were inconclusive

ACC accidents and other sudden deaths from non infectious causes 6 boys and 2 girls Five infants died from suffocation one died after 3 hours from a head injury one died from acute pontine and cerebellar hemorrhage one died suddenly from endocardial fibroelastosis

SDI sudden and unexpected death during an apparently mild infectious disease other than common cold 4 boys and no girls All four infants had suffered from acute gastroenteritis, which had lasted for ½, 1½, 2 and 5 days respectively None of the infants in this group had been considered to be seriously ill by their surroundings or had been seen by a doctor until they were found dead

RDI rapid death from infectious disease with less than 2 days duration of symptoms 3 boys and 2 girls Three infants died from pneumonia or acute tracheobronchitis one died from shock and convulsions one was believed to have died from septicemia All had high fever

DI death from infectious disease with 2 to 14 days duration of symptoms 5 boys and 5 girls Five infants died from acute gastroenteritis after 2 3 3 4 and 9 days four died from bronchopneumonia after 2 4 5 and 9 days one died from acute myocarditis and a probably viral pneumonia after 7 days of illness

Anamnestic data were taken from the clinical records, and macroscopic findings were taken from the autopsy reports

serum bilirubin in offspring of woman treated with phenobarbitone during pregnancy *Lancet II* 122 1968
6 Ramboer C Thompson R P H & Williams R Controlled trials of phenobarbitone therapy in neonatal jaundice *Lancet I* 966 1969
7 Remmer H Die Induktion arzneimittelabbauen der Enzyme im endoplasmatischen Reticulum der Leberzelle durch Pharmaka *Dtsch med Wschr* 92 2 001 1967
8 Roberts R J & Plaa G L Effect of phenobarbital on the excretion of an exogenous bilirubin load *Biochem Pharmacol 16* 827 1967
9 Trolle D Phenobarbitone and neonatal icterus *Lancet I* 251 1968
10 — Decrease of total serum bilirubin concentration in newborn infants after phenobarbitone treatment *Lancet II* 705 1968
11 Valaes T & Doxiadis S A Trial of maternal phenobarbitone medication in a population with high unspecific neonatal hyperbilirubinaemia *Abstr Ann Meeting Europ Soc Pediat Res* Interlaken Sept 14-18 1969
12 Walker W Hughes M I & Barton M Barbiturate and hyperbilirubinaemia of prematurity *Lancet I* 548 1969
13 Yeung C Y & Field C E Phenobarbitone therapy in neonatal hyperbilirubinaemia *Lancet II* 135 1969

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nation aspirated material was seen in alveoli or small bronchi in only 3 cases of SUD and in 1 case in each of the ACC SDI and DI groups

Desquamation of the bronchiolar epithelium which has been taken to indicate acute viral infection (2) was roughly estimated as the percentage of bronchiolar epithelium that had been separated from the basement membrane and the percentage of bronchiolar epithelium that had been fragmented into single cells or small groups of a few cells each The results give no evidence for an association between desquamation of bronchiolar epithelium and SUD (Table 4)

Also the following features occurred with about the same frequency in all groups Hyperemia and increased mucus secretion in the trachea and main bronchi erythrocytes inflammatory cells and edemal fluid in the lumen of small bronchi macrophages in the alveoli peribronchial lymphocyte aggregates No case of cytomegaly was diagnosed

Acute bronchiolitis was seen in 2 cases in the SUD group and in 1 case in each of the groups SDI RDI and DI

Focal collapse of the lungs had been described from macroscopic examination in about half of the SUD cases and in only 2 cases in other groups By microscopic examination, however partial diffuse or focal collapse was a common finding in all groups

Lung edema tended to be more pronounced in SUD cases than in the other groups (Table 5) and a similar though weaker tendency was seen for hyperemia of the lungs (Table 6)

Occasional intraalveolar granulocytes were seen in eight SUD infants No more than occasional intraalveolar granulocytes were also seen in 2 cases of the RDI group one of which had clinical signs of pneumonia and died 20 hours after the onset of symptoms the second RDI case was diagnosed as acute tracheobronchitis and died 30 hours after the onset of symptoms On the other hand a few intraalveolar granulocytes were also seen in 2 cases of the ACC group

Table 4 *Desquamation of bronchiolar epithelium Means of individual estimates*

	SUD (%)	ACC ( )	SDI (%)	RDI (%)	DI (%)
Portion of epithelium separated from the basement membrane	62	64	57	49	64
Portion of epithelium fragmented into single cells or small cell groups	44	33	40	[illegible]	41

Table 5 *Lung edema microscopic evaluation*

	SUD	ACC	SDI	RDI	DI
Absent	4	3	3	2	5
Slight or moderate	10	4	—	[illegible]	4
Severe	11	1	—	1	1
Total	[illegible]	8	3	5	10

Table 6 *Lung hyperemia microscopic evaluation*

	SUD	ACC	SDI	RDI	DI
Absent	4	2	2	—	4
Slight or moderate	9	3	1	3	4
Severe	12	3	—	2	2
Total	25	8	3	5	10



Fig 1 Maximum number of granulocytes per 1/4 visual field (40× objective 10× Kpl eyepieces) of lung parenchyma. SUD sudden unexpected and unexplained deaths ACC accidents and other explained non infectious sudden deaths SDI sudden unexpected deaths during acute gastroenteritis RDI deaths from infectious diseases of less than 2 days duration DI deaths from infectious diseases of 2 14 days duration The asterisks indicate 2 cases of bronchopneumonia with abscess formation and granulocyte fragmentation, in which no counts were made

Table 1 *Age distribution*

Age in months	SUD	ACC	SDI	RDI	DI
1/2–4	13	5	1	5	5
4–8	7	1	—	—	1
8–12	4	1	—	—	2
12–24	2	1	3	—	2
Total	26	8	4	5	10

Table 2 *Seasonal variation of deaths*

	SUD	ACC	SDI	RDI	DI
Jan–March	8	3	—	1	4
Apr–June	5	1	3	1	2
July–Sept	4	3	1	—	1
Oct–Dec	9	1	—	3	3
Total	26	8	4	5	10

The available histological sections from heart (39 cases) kidney (46 cases) lung (51 cases) thymus (42 cases) and spleen or lymph nodes (32 cases) were reviewed The examination of sections was made without knowledge of which diagnostic group each specimen belonged to and all histological findings were recorded according to a pre made list of relevant details In the lung sections the maximum number of granulocytes per 1/4 visual field of alveolar tissue (40× objective 10× Kpl Zeiss eye pieces) was estimated The granulocytes were counted in two or three fields selected for high cellularity and the highest count obtained in each case was recorded In the thymus sections the ratio between cortical and medullary areas was estimated Each section was projected with a magnification of 16× onto a sheet of paper and areas of cortex and medulla were drawn and measured with a planimeter

ANAMNESTIC DATA

The age distribution is shown in Table 1 Most cases of SUD occur during the first 4 months of life, and only 2 cases were seen during the second year

Seasonal variation is similar for SUD and for deaths from acute infections with alarming symptoms (RDI DI) both occurring with highest frequency during winter months (Table 2)

Ten of the SUD infants had shown trivial symptoms of upper respiratory tract infections in the days prior to death, and three more had been more restless than usual

Thirteen SUD infants were said to have been found with bedclothes covering their faces There was a striking difference in age between those who had their faces covered and the rest of the SUD group (Table 3) No infant younger than 3 months of age was said to have had its face covered whereas nearly all SUD infants older than 4 months of age were said to have had their faces covered

Nutrition is known in only seven SUD infants, six had been artificially fed and one had been at least partially nourished by the mother

AUTOPSY FINDINGS

Heart There was 1 case of acute myocarditis in the DI group 1 case of endocardial fibroelastosis in the ACC group and 1 case of ostium atrioventriculare commune in a mongoloid infant in the RDI group The remaining cases had no sign of heart disease or abnormality

Kidneys Fibrosis and degeneration of scattered glomeruli as have been described in SUD (3 15) were seen in all groups and were no more common in SUD than in infants that died from known causes

Respiratory organs Petecchial hemorrhages in the lungs and other thoracic viscera were seen in some cases of all groups Microscopic petecchiae on chest organs were even more frequent in the ACC group (7 of 8) than in the SUD group (15 of 26)

Aspiration of ventricular contents to the respiratory passages appeared not to be an important factor in SUD By microscopic exami

Table 3 *Covering of the face and age distribution in SUD*

	Ages in months/days
Infants said to have had their faces covered	3/0 3/17 4/28 5/18 6/19 6/21 6/25 7/9 8/23 9/5 10/4 10/28 13/30
Infants with no mentioning of covering of the face	0/16 1/6 1/22 1/29 2/4 2/8 2/10 2/27 3/3 3/9 3/16 4/15 19/26

among different groups Nor are varying degrees of lung hyperemia responsible for the variation of granulocyte counts Four SUD infants with no hyperemia of the lungs had 17 ±11 granulocytes (mean ±1 S D) nine SUD infants with slight or moderate hyperemia had 26±22 granulocytes and twelve SUD infants with severe hyperemia had 25±19 granulocytes per 1/4 visual field

For comparison with the findings in the lungs the number of granulocytes in ten randomly chosen renal glomeruli was counted in each case of the SUD ACC and RDI groups in which kidney sections were available (35 cases) Contrary to the findings in the lungs the average numbers of glomerular granulocytes showed no obvious difference among the three groups

Colonies of bacteria were seen in the lung parenchyma in seven SUD cases one of which had microscopic evidence of aspiration In the other groups colonies of bacteria were seen in one SDI case in which microscopic evidence of aspiration was also present in two DI cases with fully developed bronchopneumonia and in one DI case with a possible imminent pneumonia No colonies of bacteria were seen in ACC and RDI cases Bacteriological cultures were made from only two of the SUD cases in which bacterial colonies were seen in the histological sections both showed the presence of *Staphylococcus aureus* Bacteriological examination from the lungs of two other SUD cases showed *E coli* in one and normal bronchial flora in the other

Thymus The mean weight of the thymus was slightly higher in the ACC group (33 g) than in the SUD group (26 g) but the difference is not statistically significant The ratio of cortical areas to medullary areas in thymus sections was practically identical in ACC and SUD groups In the SDI RDI and DI groups both the weight and the cortico-medullary ratio tended to be lower although in most cases in which the illness had lasted for less than 2 days the thymus parameters were within the range of the ACC and SUD cases

Spleen and lymph nodes Neither spleen weight nor the number and size of germinal centers of spleen and lymph nodes or their content of fragmented cell nuclei showed any significant difference among the groups

DISCUSSION

None of the findings in this material could justify definite conclusions about the causal mechanisms of SUD As in other reports (2 9) aspiration of ventricular contents to the respiratory passages appear to be a possible mechanism only in a small minority of cases When seen together several of the present observations could be taken to indicate that bacterial pneumonias that reach only to the initial phase of the disease could be the cause of many cases of SUD either as the single determinant factor or possibly in conjunction with other factors predisposing to sudden death

Most remarkable is the finding of high numbers of neutrophilic granulocytes in the alveolar septa in the older SUD infants and the relation between granulocyte numbers and age in the SUD group The observations may mean that the defense mechanisms in SUD infants under 4 months of age are so poorly developed that virulent infections lead to death before the infants have been able to mobilize their granulocyte reaction whereas after the age of 4 months this reaction is gradually strengthened until at the end of the first year the defence mechanisms are usually good enough to prevent the occurrence of SUD In this connection it may be noticed that the maximum SUD frequency at 3 months of age (6) coincides with the age at which serum concentrations of IgG immunoglobulins reach minimum levels (1 4)

It is very rare that older children or adults die during the first day of a bacterial pneumonia but when this occurs the picture is different from that commonly diagnosed as pneumonia by the pathologist In the first day of a lobar pneumococcal pneumonia hyperemia and edema are the main features and only a

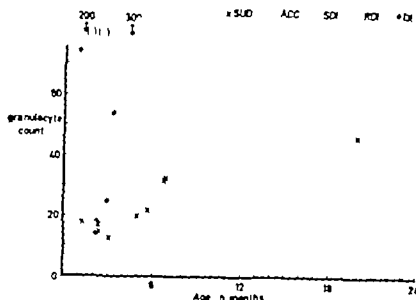


Fig 2 Same data as in Fig 1 related to the age of the infants Abbreviations and asterisks as in Fig 1

Granulocytes in the alveolar septa were much more common than intraalveolar granulocytes the great majority being neutrophils Fig 1 shows the counted maximum numbers of granulocytes both intraalveolar and septal, per 1/4 visual field of lung parenchyma The normal number that is the number of granulocytes that can be expected when no lung disease is present can best be estimated from the findings in the infants who died from obvious accidents With the exception of one infant the granulocyte counts of the ACC group are below twenty The one exception is an infant whose allocation to the ACC group or to the SUD group was questionable Thus normal granulocyte counts are tentatively taken to be below twenty per 1/4 visual field

In the SUD group granulocyte counts range from 3 to 68 and in ten SUD cases the counts are higher than 20 In the groups of recognized infectious diseases (SDI RDI DI) all cases that had signs or symptoms of respiratory infections except for the case of probable viral pneumonia in the DI group had granulocyte counts higher than 20 This includes 1 case of gastroenteritis with 65 granulocytes in the SDI group, in which a fibrinous pleuritis was also present A second case of gastroenteritis in the DI group had a granulocyte count of 45 No further evidence of respiratory infection is known in this case but the presence of an imminent complicating pneumonia remains a possible explanation In the RDI group the granulocyte counts above 20 were 25 in an infant with clinical sepsis 30 in an infant with marked acute bronchiolitis and tracheobronchitis 54 in a mongoloid infant with ostium atrioventriculare commune and clinical signs of pneumonia about 200 in an infant with fully developed bronchopneumonia Granulocyte counts below 20 were recorded in the SDI group in 2 cases of gastroenteritis, in the DI group in 4 cases of gastroenteritis and in the 1 case of probable viral pneumonia and in the RDI group in 1 case with extensive lung hemorrhage

There is an obvious association between age and granulocyte counts in the SUD group as shown in Fig 2 The granulocyte counts are low and less than 20 in all SUD infants younger than 4 months of age In older SUD infants there is a gradual increase of granulocyte counts with age No similar association between age and granulocyte counts is evident in the other groups It should be recalled that the ACC case with the highest granulocyte count had a doubtful group allocation and might possibly have been grouped with the SUD cases

There is no consistent association between granulocyte counts and resuscitation, either within the SUD group or in the comparison

12 Parish W E Hall L W & Coombs R R A The effect of anaesthesia on anaphylaxis in Guinea pigs *Immunology* 6 462 1963
13 Parish W E Richards C B France N E & Coombs R R A Further investigations on the hypothesis that some cases of cot-death are due to a modified anaphylactic reaction to cow's milk *Int Arch Allerg* 24 215 1964
14 Peterson R D A & Good R A Antibodies to cow's milk proteins — their presence and significance *Pediatrics* 31 209 1963
15 Stowens D Callahan E L & Clay J Sudden unexpected death in infancy A new hypothesis of cause *Clin Pediat* (Phila) 5 243 1966
16 Valdes Dapena, M A Sudden and unexpected death in infancy A review of the world literature 1954 1966 *Pediatrics* 39 123 1967
17 Woolley P V Jr Mechanical suffocation during infancy A comment on its relation to the total problem of sudden death *J Pediat* 26 572 1945
18 Wright G Payling & Heard B E The lungs including the trachea and bronchi and the pleura in G Payling Wright and W St C Symmers (eds) *Systemic pathology* Longmans, London 1966 p 333

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few erythrocytes and granulocytes are present in the alveolar fluid It is not until the second day that granulocytes appear in appreciable numbers in the alveoli (11 18) Hyperemia and edema of the lungs were prominent in most of the SUD cases in the present material and a few intraalveolar granulocytes were present in one third Similar findings were made in some of the infants who died after a very brief but recognized history of clinical pneumonia or acute tracheobronchitis

Other evidence for the role of infections in SUD are the frequent observation of bacterial colonies in histological lung sections in this material, and the high frequency of preceding symptoms of upper respiratory tract infections and the seasonal variation parallell to that of respiratory infections as have been common findings in other studies as well (2, 5 6 9)

The reason why pathogenic bacteria have been demonstrated so infrequently in most series of SUD autopsies may lie in the conditions under which bacteriological cultures have been taken and grown Johnstone & Lawy (10) paid special attention to this point, and by setting up cultures from bronchial secretions under a set of different culture conditions they were able to demonstrate the presence of pathogenic bacteria in 37 out of 55 cases of SUD

Covering of the face with bedclothes was the rule in SUD infants older than 4 months and was absent from SUD infants younger than 3 months in the present study A possible explanation is that under a feeling of cold that accompanies a rapid rise in body temperature, covering of the face is actively sought for by those infants who are old enough to be able to do so Thus, covering of the face with bedclothes may not be a cause of death but rather a side effect of a disease that leads to SUD

SUMMARY

Autopsy findings in 26 typical cases of sudden and unexpected infant death (SUD) are reported and compared to findings in non infectious sudden deaths and deaths from acute infectious diseases Lung edema and hyperemia were on the average more pronounced in SUD than in the controls Bacterial colonies in histological lung sections were seen in seven SUD cases in the control groups such findings were generally associated either with bronchopneumonia or with microscopic evidence of aspiration Increased numbers of neutrophilic granulocytes were seen in the lung alveolar septa in SUD infants over 4 months of age, and occasional intraalveolar granulocytes were present in one third of the SUD cases Covering of the face with bedclothes was the rule in SUD infants older than 4 months and was absent from SUD infants younger than 3 months of age It is suggested that bacterial pneumonias that reach only to the initial phase of the disease may be the cause of many cases of SUD and that covering of the face with bedclothes may be actively sought for by the older SUD infants under a feeling of cold accompanying a rapid rise in body temperature

REFERENCES

1 Allansmith M R McClellan B H & Butterworth M Individual patterns of immunoglobulin development in ten infants *J Pediat 75* 1231 1969
2 Banks A L An enquiry into sudden death in infancy *Monthly Bull Minist Health* (London) *17* 182 1958
3 Barrett A M Sudden death in infancy *in* D Gairdner (ed) *Recent advances in paediatrics* J & A Churchill Ltd London 1954 p 301
4 Berg T The immunoglobulin development during the first year of life A longitudinal study *Acta Paediat Scand 58* 229 1969
5 Cooke R T & Welch R G A study in cot death *Brit Med J 2* 1549 1964
6 Geertinger P *Pludselig uventet spædbarnsdød i København* Munksgaard København 1966
7 — Pludselig uventet spædbarnsdød *Lakartidningen 66* 677 1969
8 Giertsen J C Plutselig død hos spebarn *Nord Med 77* 825 1967
9 Jacobsen T & Voigt J Sudden and unexpected infant death II Result of medico legal autopsies of 356 infants aged 0–2 years *Acta Med Leg Soc* (Liege) *9* 133 1956
10 Johnstone J M & Lawy H S Role of infection in cot deaths *Brit Med J 1* 706 1966
11 Millard M Lung pleura and mediastinum *in* W A D Anderson (ed) *Pathology* 5th ed Mosby St Louis 1966 p 686

12 Parish W F Hall L W & Coombs, R R A The effect of anaesthesia on anaphylaxis in Guinea pigs *Immunology* 6 462 1963
13 Parish W E Richards C B France N E & Coombs R R A Further investigations on the hypothesis that some cases of cot-death are due to a modified anaphylactic reaction to cow s milk *Int Arch Allerg* 24 215 1964
14 Peterson R D A & Good R A Antibodies to cow s milk proteins — their presence and significance *Pediatrics* 31 209 1963
15 Stowens D Callahan E L & Clay J Sudden unexpected death in infancy A new hypothesis of cause *Clin Pediat* (Phila) 5 243 1966
16 Valdes Dapena M A Sudden and unexpected death in infancy A review of the world literature 1954–1966 *Pediatrics* 39 123 1967
17 Woolley P V Jr Mechanical suffocation during infancy A comment on its relation to the total problem of sudden death *J Pediat* 26 572 1945
18 Wright, G Payling & Heard, B E The lungs including the trachea and bronchi and the pleura in G Payling Wright and W St C Symmers (eds) *Systemic pathology* Longmans, London 1966 p 333

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THE ELECTROLYTE AND PROTEIN CONTENTS AND OUTPUTS IN DUODENAL JUICE AFTER PANCREOZYMIN AND SECRETIN STIMULATION IN NORMAL CHILDREN AND IN PATIENTS WITH CYSTIC FIBROSIS [1]

G ZOPPI D H SHMERLING [2] D GABURRO and A PRADER

From the Clinica Paediatrica University of Ferrara Ferrara Italy and the Kinderspital University of Zurich Zurich Switzerland

The concentration of electrolytes and protein in duodenal juice of various animals and in man is well known (1 2, 7 8 9, 11, 14 17 18) Yet systematic studies on total outputs and secretion patterns of ions and protein after hormonal stimulation with pancreozymin and secretin are lacking especially so in infants and children (5 6)

This problem assumes a particular importance in disorders of exocrine pancreatic secretion, e g in cystic fibrosis of the pancreas (6) since in this disease a generalized disturbance of water and electrolyte movement in exocrine tissues and particularly in the exocrine pancreas has been recently assumed to play an important role (6 10)

In order to study *such a hypothesis* duodenal contents have to be sampled as quantitatively *as possible* after hormonal *stimulation* of the exocrine pancreas, so that outputs and secretion rates can be calculated This was attempted in the investigations reported here

MATERIAL AND METHODS

Subjects studied

17 children aged 9 months to 13 years have been studied 12 did not present any evidence of exocrine pancreatic insufficiency 5 suffered from cystic fibrosis (CF) of the pancreas as demonstrated by elevated sweat electrolytes pulmonary involvement and steatorrhoea

Methods

Duodenal intubation A triple lumen double balloon tube was placed in the duodenum under fluoroscopic control as described previously in details (5 18) Pancreozymin and secretin (Boots) were given intravenously (2 I U /kg body weight each) and the duodenal content sampled in 10 min periods twice after pancreozymin and three times after secretin

Laboratory procedures Immediately after sampling the volume and pH of each fraction were measured and bicarbonate concentration determined according to Lagerlöf (12) Sodium and potassium were assayed by flame spectrophotometry total calcium according to Diehl et al (4) and magnesium as described by Thiers (15) Protein was estimated by the method of Lowry et al (13)

Proteinase lipase and α amylase activities of duodenal juice were also tested as described previously (5) *as control of pancreatic activity* Electrolytes were assayed and reported here only in patients in whom at least some pancreatic enzyme activity was detectable *Cases* with no enzyme activities were excluded

Fluid volume enzyme activities protein and electrolytes were expressed as total outputs per kg body weight and per 50 min after *hormonal stimulation* secretion rates of fluid volume protein and electrolytes expressed per kg and per min were calculated separately for the post pancreozymin and the post secretin periods

RESULTS

Table 1 shows the total outputs of enzyme activities both in controls and in patients ex

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Table 1 *Total outputs of peptidases α amylase and lipase in duodenal juice of normal controls and patients with cystic fibrosis of the pancreas* (CF) (*mean and range/kg body weight 50 min after pancreozymin and secretin stimulation*)

Case	Chymotrypsin (µg)	Trypsin (µg)	Carboxypeptidase A (I U 10³)	α Amylase (I U)	Lipase (I U)
Controls					
n = 12 Mean (range)	880 (270–1 900)	765 (215–2 000)	712 (185–2 200)	651 (160–2 150)	1 464 (350–5 000)
Patients					
1	0	0	0	28	0
2	0	2	0.5	6	41
3	0	0	0	270	0
4	36	41	85	73	341
5	0	0	0	8	0

pressed per kg and per 50 min after hormonal stimulation

In Table 2 total outputs of fluid volume protein and electrolytes per kg and per 50 min after hormonal stimulation in normal controls and in patients are reported The values of fluid volume and electrolytes appear to be strongly lowered in CF patients As previously described (18) protein output is also decreased but the difference is not statistically significant Expressed as percentages of the normal controls (Table 2) the secretion of fluid volume sodium potassium and calcium in CF patients is reduced to the same amount Bicarbonate is reduced much more whereas magnesium and protein much less than the fluid volume

Table 3 shows the secretion rates of fluid volume (expressed in ml per kg and per min) of protein and of electrolytes (µg per kg and per min) in normal controls and in patients after pancreozymin and Table 4 those after secretin stimulation A comparison of the secretion rates after pancreozymin and after secretin in normal controls (Fig 1) shows that the maximal secretion rate of sodium and bicarbonate is reached after secretin whereas that of protein calcium and magnesium is reached after pancreozymin Secretion rates of potassium and

Table 2 *Total outputs of fluid volume protein and electrolytes in duodenal juice of normal controls and patients with cystic fibrosis of the pancreas* (*CF*) (*mean ± 1 S D mg/kg body weight 50 min after pancreozymin and secretin stimulation*)

	Normal controls	CF patients	CF patients in % of normals
n	1	5	
Volume (ml kg 50 min)	3.9 ± 1.6	0.8 ± 0.4	70
Protein	15.4 5.5	9.7 ± 8.6	63
Bicarbonate	15 ± 7	1.4 ± 1.5	9
Sodium	13.9 ± 6.4	2.5 ± 0.3	18
Potassium	0.8 _ 0.3	0.3 ± 0.07	25
Calcium	0.1 ± 0.05	0.04 ± 0.07	33
Magnesium	0.05 ± 0.01	0.03 _ 0.02	60

Table 3 *Secretion rates of fluid volume protein and electrolytes in normal controls and in patients with cystic fibrosis of the pancreas* (*CF*) (*mean ± 1 S D µg/kg body weight 1 min after pancreozymin stimulation*)

	Normal controls	CF patients	CF patients in % of normals
n	12	5	
Volume (ml/kg min)	0.06 ± 0.01	0.02 ± 0.004	33
Protein	599 ± 107	[illegible]08 ± 133	35
Bicarbonate	167 ± 73	15 ± 24	9
Sodium	240 ± 101	65 ± 90	27
Potassium	16.8 ± 6.5	5.6 ± 6.4	33
Calcium	3.6 ± 1.4	0.9 ± 0.8	[illegible]
Magnesium	1.2 ± 0.3	0.8 ± 0.7	71

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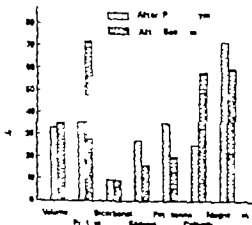


Fig. 2. Comparison of secretion rates after pancreozymin and secretin in cystic fibrosis patients (% of mean values found in normal controls).

are similar. Bicarbonate is reduced much more and protein and magnesium much less than the fluid volume. This means that the concentrations of protein and magnesium in duodenal juice of CF patients after hormonal stimulation is relatively higher than the concentration of sodium and potassium.

The mechanisms of secretion of water and of various electrolytes seem therefore to be altered in different degrees.

Secretion rates

In normal controls the secretion rates of fluid volume after pancreozymin and after secretin (Fig. 1) seem not to be significantly different after both hormonal stimuli. Pancreozymin is known to stimulate the acinar cells of the pancreas whereas secretin stimulates the centroacinar and the intercalated duct cells. The secretion patterns of water in both compartments of the gland are therefore probably similar.

The secretion rates of sodium, potassium and magnesium in CF patients was shown to be more strongly reduced after secretin, that of calcium and protein after pancreozymin and that of fluid volume and bicarbonate similarly reduced after both hormonal stimulations. In patients with proved cystic fibrosis having exocrine pancreatic insufficiency but some detectable enzymatic activities in the duodenal juice not only the secretin-dependent secretion produced by the centroacinar and the intercalated duct cells is greatly decreased but also the pancreozymin-dependent secretion from the acinar cells.

The non-selective destruction of the gland in the disease, which in our patients (all presenting with steatorrhea) must have been quite extensive, leads to a disorganized pattern of defects in the secretion rates. There is no evidence for a more accentuated defect in either the pancreozymin or the secretin-dependent phase of secretion. On the other hand there is a non-parallel reduction of fluid volume, sodium and bicarbonate after hormonal stimulation in CF patients. Since in exocrine cells the transport of water and sodium is passive while the transport of bicarbonate may be active (10) it seems likely that in cystic fibrosis both the passive and the active transport mechanisms of ions across the cell membrane of the exocrine pancreas are altered.

SUMMARY

The total pancreatic outputs and the secretion rates of fluid volume, protein, bicarbonate, sodium, potassium, calcium and magnesium were studied after pancreozymin and secretin stimulation in 12 control children and in 5 patients with cystic fibrosis of the pancreas (CF). Duodenal contents were collected through a double balloon, triple lumen rubber tube, thus avoiding contamination by gastric juice and distal losses. In CF patients compared to the normal controls decreased outputs of fluid volume, protein and electrolyte were found.

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	Normal controls	CF patients	CF patients in of normals
n	12	5	
Volume (ml/kg/min)	0 07±0 02	0 02±0 008	28
Protein	262±143	202±220	72
Bicarbonate	436±209	36±46	9
Sodium	300±156	48 5±16 9	16
Potassium	15 3±5 9	3 1±0 5	20
Calcium	1 2±0 8	0 7±0 3	58
Magnesium	0 8±0 4	0 5±0 4	59

fluid volume did not show any significant difference between the post pancreozymin and the post secretin periods

In CF patients secretion rates of fluid volume protein and electrolytes are lowered In Fig 2 secretion rates after pancreozymin and after secretin expressed as percentages of mean values found in normals are compared The secretion rate of sodium potassium and magnesium is more strongly reduced after secretin, that of protein and calcium after pancreozymin and that of fluid volume and bicarbonate is similarly reduced after both hormonal stimulations

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Total Outputs

With the method described above we could determine with a good approximation the total pancreatic outputs of fluid volume, protein and electrolytes after hormonal stimulation with pancreozymin and secretin The double balloon tube used here—as previously discussed (5 18)—permits as far as possible a quantitative sampling of duodenal contents without any contamination with gastric juice and distal losses

Duodenal juice is often bile stained after pancreozymin stimulation since pancreozymin (Boots) shows a cholecystokinin activity which is presumably quite constant This fact, which is negligible in the case of enzyme determinations, may play a role in the case of electrolytes Bile contamination would in such a case lead to a similar contribution of electrolytes to the juice after pancreozymin stimulation in both controls and patients As the electrolyte content of bile is relatively low (3 6) such a contamination would probably not alter the results significantly

In the CF patients studied here, the pancreatic secretion was strongly diminished but not completely absent as evidenced by the detectable enzyme activities

In CF patients the reduction of total outputs of fluid volume sodium potassium and calcium

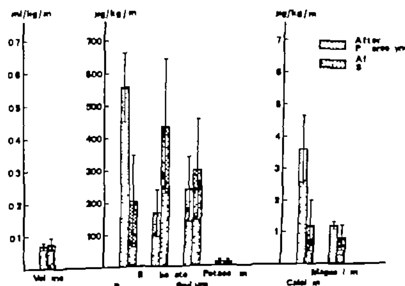


Fig 1 Comparison of the secretion rates after pancreozymin and secretin in normal controls (secretion/kg body weight/min)

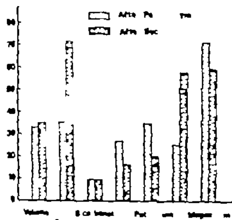


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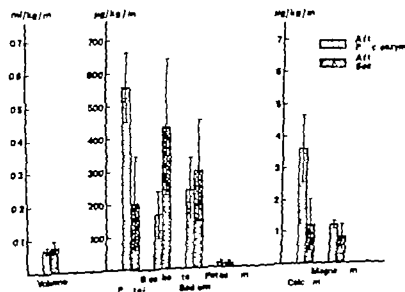


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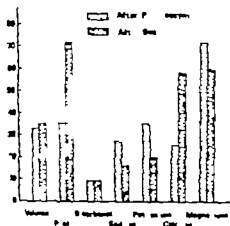


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The possible role of a disturbance of the tubular and the acinar secretion of the pancreas in the pathogenesis of cystic fibrosis is discussed

REFERENCES

1 Birnbaum D & Hollander F Relation between bicarbonate concentration and rate of canine pancreatic secretion *Amer J Physiol 209* 966 1965
2 Case R M Harper A A & Scratcherd T The secretion of electrolytes and enzymes by the pancreas of the anaesthetized cat *J Physiol 201* 335 1969
3 Cooke R E & Crowley L G Replacement of gastric and intestinal fluid losses in surgery *New Eng J Med 246* 637 1952
4 Diehl H & Ellingboe J L Indicator for titration of calcium *in* presence of magnesium using disodium dihydrogen ethylendiamine tetraacetate *Anal Chem 28* 882 1956
5 Hadorn B Zoppi G Shmerling D H Prader A McIntyre I & Anderson C M Quantitative assessment of exocrine pancreatic function in infants and children *J Pediat 73* 39 1968
6 Hadorn B Johansen P G & Anderson C M Pancreozymin secretin tests of exocrine pancreatic function in cystic fibrosis and the significance of the results for the pathogenesis of the disease *Canad Med Ass J 98* 377 1968
7 Hinsky J Calcium content of duodenal juice *Amer J Dig Dis 12* 725 1967
8 Henriksen F W Effects of pancreozymin on the canine pancreatic secretion of fluid and bicarbonate *Scand J Gastroent 3* 637 1968
9 Henriksen F W & Worning H The relation between the concentrations of bicarbonate and protein in the pancreatic juice in the dog *J Physiol 187* 285 1966
10 Johansen P G Anderson C M & Hadorn B Cystic fibrosis of the pancreas A generalized disturbance of water and electrolyte movement *in* exocrine tissues *Lancet 1* 455 1968
11 Janowitz, H D & Dreiling D A The pancreatic secretion of fluid and electrolytes. In *The exocrine pancreas normal and abnormal function* Ciba Foundation, Churchill London 1962 p 115
12 Lagerlof H O Pancreatic function and pancreatic disease studied by means of secretin *Acta Med Scand* Suppl *1 8* 1 1942
13 Lowry O H Rosenbrough H Farr A L & Randall R J Protein measurements with the *Folin phenol reagent J Biol Chem 193* 265 1951
14 Schmid W C Phillips S F & Summerskill, W H J Jejunal secretion of electrolytes and water in nontropical sprue *J Lab Clin Med 73* 773 1969
15 Thiers R E Magnesium (fluorimetric) In *Standard methods of clinical chemistry* vol 5 Academic Press New York and London 1965 p 131
16 Thureborn E Human hepatic bile composition changes due to altered enterohepatic circulation *Acta Chir Scand* Suppl *303* 1 1962
17 Zimmerman M J Dreiling D A Rosenberg R & Janowitz, H D Secretion of calcium by the canine pancreas *Gastroenterology 52* 865 1967
18 Zoppi G Hitzig W H Shmerling D H Pluss H Hadorn B & Prader A Protein content and pancreatic enzyme activities of duodenal juice in normal children and in children with exocrine pancreatic insufficiency *Helv Paediat Acta 23* 577 1968

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CASE REPORT

RAPID GROWTH AND CENTROLOBULAR EMPHYSEMA AFTER STENOSING TRACHEITIS AND CONTINUOUS EXPOSURE TO LOW CONCENTRATIONS OF OXYGEN

CHARLES P BIEBER ROBERT C ROSAN WILLIAM H NORTHWAY JR
PENELOPE CAVE SMITH WILLIAM J DAILY and MICHAEL M BRAND

From the Departments of Pathology Radiology Anesthesiology and Pediatrics Stanford Medical School Palo Alto California USA

Lobular emphysema of the newborn which was not mentioned in pediatric textbooks prior to the study of Wilson & Mikity in 1960 (21) now occurs in near-epidemic form (13 18 19) Robertson *et al* were the first to associate the triad of hyaline membrane disease prematurity and oxygen toxicity with cystic emphysema (16) Although it is clear that no one of these factors is essential (6) premature birth (6 19) and low birth weight (6 14) propitiate oxygen toxicity It was early suggested that the emphysema of oxygen toxicity was largely caused by bronchiolar lesions (11 13 17) an opinion later corroborated by Hawker *et al* (7) and Gaillard (6) Oxygen is not unique in this respect viral agents which cause neonatal bronchiolitis may also cause emphysema (1) The unique cellular structure of the neonatal bronchiole may render the premature infant vulnerable to emphysema after bronchiolitis (10)

With these thoughts we present the case of a very small premature infant who was long exposed to low concentrations of supplementary oxygen but who also had retrolental fibroplasia pneumonia, and stenosing tracheitis He is the first infant with centrolobular emphysema who died when his cardiopulmonary condition was almost within normal *clinical* limits

This investigation was supported by USPHS-NIH grant FR 81 and the Council for Tobacco Research—USA grant 644

CASE REPORT

Clinical history M C was an 846 g male whose gestational age was estimated at 28 weeks and neurological age at 32 weeks The maternal and family history is not pertinent The 1 minute Apgar score was 1 Although he had respiratory pauses mild retractions slight cyanosis and infrequent grunting for the first 12 hours his chest radiographs were normal He ceased grunting after 12 hours At 1 days he was transferred to our hospital During the next 8 days he had numerous short bouts of apnea These were treated with physical stimulation and 100 oxygen delivered briefly by bag and mask He received a total of about 2100 hours of oxygen in concentrations exceeding 40 during the first 116 days of life as shown in Fig 1

When he became listless on the 8th day cultures were obtained These demonstrated *Pseudomonas* in the cerebrospinal fluid conjunctivae and stool Despite treatment with Kanamycin® and Colistin® he developed radiographic infiltrates of both lungs and an exacerbation of the apneic episodes Therefore he was maintained in a Bennett positive pressure ventilator for the next 5 / days (20) This therapy included endobronchial suction each hour to remove sticky secretions (12) By the 12th day the right lung field was radiographically opaque and there was a pseudomonal tracheitis Cytological examination of his endobronchial secretions during the exposure to oxygen via the ventilator showed an increasingly bizarre atypia a marked diminution of the ciliated

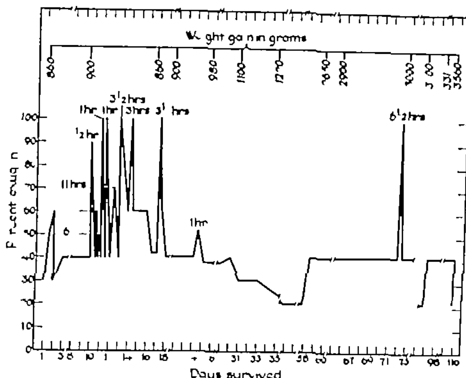


Fig 1 The administration of more than 2100 hours of oxygen at concentrations of 40 or more is shown graphically There is one period of assisted ventilation beginning on day 10 and extending 5 1/ consecutive days during which a total of 25 / hours of oxygen was administered at concentrations exceeding 60 via a Bennett machine The excellent weight gain which begins on day 21 was accompanied by normal growth and development throughout the remainder of the infant's life of 4 / months The major problem up to day 12 was apnea and extreme prematurity to day 18 pneumonitis to day 116 and thereafter tracheitis

cell population and only moderate inflammatory exudate (12)

During the next 2 weeks he followed an accelerating course of progressive radiological clinical and cytological improvement (12) even though he had tracheitis and recurrent atelectases When he was 10 weeks old his birth weight had tripled At this time the second clinical stage of retrolental fibroplasia (15) was initially observed The records showed that the P_{O_2} of aortic blood had exceeded 160 mm Hg only once

During his 14th week an elective tracheostomy was performed for a stenosis which had enveloped the entire trachea Despite divers complications of stomal infection pneumothorax pneumomediastinum and dislocation of the prosthesis he thrived In the 25th week he was sent home with a permanent tracheostomy He weighed 3950 g

He was readmitted for study 2 weeks later His physician noted an alert well hydrated infant with somewhat excessive ventilatory motions but without retractions rales or ronchi The radiologist found only a few strand like shadows in the right upper lung field The urinalysis and hemogram were completely normal He weighed 4100 g On the 2nd hospital day he was discovered lifeless in his crib with the endotracheal tube dislodged

Autopsy Significant lesions were found only in the trachea lungs eyes and brain Both lungs were stiff and knobby *Dilated airspaces were easily seen* through the glistening pleurae and on cut section throughout the lung The lymphatics were prominent While the middle lobe occupied two thirds of the *right pleural* cavity the right upper lobe was atrophic bosselated and focally atelectatic Although collapsed at autopsy the left lung resembled the right when inflated with fixative but its lobes were in normal



Fig 2 The fixed lung is a faithful replica of the lung *in situ* at autopsy The many small grey spots are emphysematous subpleural alveolar ducts On cut section they were irregularly distributed throughout all lobes The contracted intersegmental markings are most often associated with hyperplastic lymphatics The lobular bronchioles were dilated and ectatic but there were no mucus plugs Although the pulmonary arteriolar system was mildly hyperplastic the heart was within normal limits for size weight and thickness of ventricular myocardium

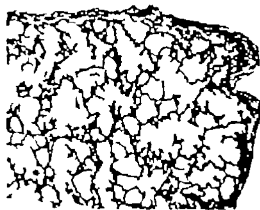


Fig 3 The alveolar ducts are capacious and the alveolar sacs have a simplified appearance because the interalveolar septae are short and blunt Even partly collapsed airways do not have a normal delicately septate pattern There is a small degree of interstitial and pleural fibrosis The irregularity of the pattern and the juxtaposition of small and large alveolar ducts is characteristic

proportion The dilated air spaces were histologically identified as distended respiratory ducts associated with ectatic bronchioles and stubby elastin rich alveolar septae This centrolobular emphysema was accompanied by a feeble interstitial fibrosis involving delicate fibrils rather than cells There was a muscular hypertrophy of pulmonary arterioles and fragmentation of elastin in large arteries The trachea though remarkably cicatrized showed little acute inflammation The striking gross lung features are shown (Fig 2) and the histology (Fig 3)

The brain weighed 390 g There was some abnormal infratentorial firmness and there was correspondingly (3 4) a marked histological fibrillary gliosis in the optic nerves and chiasm periaqueductal grey matter median longitudinal fasciculus raphe cerebral peduncles pyramids white matter of the medulla and the anterior and lateral columns of the spinal cord In the areas of gliosis there was often an increase in the number of capillaries No part of the brain demonstrated neuronal loss or necrosis In the retinae there was gliosis of the inner plexiform layer and vasoproliferation in the temporoanterior quadrant of the globes

DISCUSSION

The autopsy revealed a surprising degree of emphysema which had evaded careful clinical and radiological study and had not prevented rapid growth This is not Wilson-Mikity disease (21) nor pulmonary dysmaturity (2) syndromes which lack the combination of viscid secretions and atypical cytology followed by vascular and bronchiolar lesions with centrolobular emphysema of this case That combination does occur in oxygen toxicity (6 7 11 12 13) and perhaps other forms of neonatal bronchiolitis

Other causes of airway obstruction in this infant were the pseudomonas infections of lungs and trachea and the tracheal scar While recognizing the association of *Pseudomonas* with emphysema in cystic fibrosis we find no references to lobular emphysema after bacterial pneumonia of newborns without cystic fibrosis The possibility of tracheitis is more challenging since most pulmonary resistance is classically in the large airways What might happen to a rapidly growing lung during severe tracheal obstruction is purely conjectural for there are no studies on the subject A priori tracheal obstruction does not explain proximal lobular emphysema nor pulmonary vascular change The possibility of the lesion of the mechanical ventilator must be considered but this is supposed to be interstitial rather than bronchiolar (9) Viral disease is also a possibility (1)

If the disease is due to oxygen intoxication it has occurred at lower concentrations and over a longer course than heretofore considered usual Nevertheless this possibility remains

The brain lesions are classic for chronic hypoxia of infancy and are basically a disturbance of white rather than grey matter (3 4) On the other hand the retrolental fibroplasia was probably related to a very early and short period of hyperoxia (14) Indeed it would be interesting to know if the effects of oxygen on retinal vasculature (14) and pulmonary vasculature (8) were similar

SUMMARY

An 846 g newborn survives critical apnea pseudomonad pneumonia and stenosing tracheitis and thrives during 3 months of exposure to 40 oxygen When he dies unexpectedly during an excellent convalescence his lungs show a surprisingly advanced centrolobular emphysema The history excites skepticism about the clinical evaluation of silent

lungs of newborns and demonstrates how remarkable a disparity may exist between ventilatory function and morphological damage in newborns The possibility for contributory etiological roles is discussed for oxygen therapy tracheal obstruction and rapid growth but not for pneumonia The case recalls the disturbing increase in neonatal emphysema since 1960 Restricted use of the term Wilson–Mikity disease is urged while generic use of the term lobular emphysema, is condoned

REFERENCES

1 Adhikari P K Rao N V & Ferguson C A Chronic obstructive lung disease following viral bronchopneumonia *Pediat Res* 2 22 1968

2 Baghdassarian O M Avery M E & Neuhauser E B D A form of pulmonary insufficiency in premature infants Pulmonary dysmaturity? *Am J Roentgenol* 89 1020 1963

3 Brand M M Neuropathological observations on the respiratory distress syndrome of the new born in *Drugs and Poisons in Relation to the Developing Nervous System* U S P H S Pub No *1791 1967 p 192 ff*

4 Brand M M & Bignami A The effects of chronic hypoxia on the neonatal and infantile brain *Brain* 92 233 1969

5 Cleland R Personal communication to author (R C R) 1966

6 Gaillard J L J Pathologisch anatomische bevindingen bij kunstmatig beademde pasgeboren in *Boerhaave Cursussen voor Voortgezet Medisch Onderwijs Fysiologie en Pathologie in de Perinatale Periode* Rijksuniversiteit Leiden Nederland 1969 pp 34 35

7 Hawker J M Reynolds E O R & Taghizadeh A Pulmonary surface tension and pathological changes in infants dying after respirator treatment for severe hyaline membrane disease *Lancet II* 75 1967

8 Kistler G S Caldwell P R B & Weibel Development of the fine structural damage to alveolar and capillary lining cells in oxygen poisoned rat lungs *J Cell Biol* 33 605 1967

9 Liebow A A in A A Liebow & D E Smith (eds) *The Lung* Williams & Wilkins Baltimore 1968 p 332 ff

10 Lauweryns J M & Rosan R C The unit lobule A revised concept of the neonatal lung *Abstr 2nd Europ Congr Perinatal Med* In press

11 Northway W H Jr & Rosan R C Radiographic features of pulmonary oxygen toxicity in the newborn bronchopulmonary dysplasia *Radiol 91 49 1968*

12 — Oxygen therapy hazards in the neonate *Hosp Pract 4 59* 1969

13 Northway W H Jr Rosan R C & Porter D Y Pulmonary disease following respirator therapy of hyaline membrane disease Bronchopulmonary dysplasia *New Eng J Med 276 357 1967*

14 Northway W H Jr Rosan R C Shahinian L Jr Castellino R A Gyepes M T & Durbridge, T Radiologic and histologic investigation of pulmonary oxygen toxicity in newborn guinea pigs *Invest Radiol 4 148 1969*

15 Patz A The role of oxygen in retrolental fibroplasia *Transact Am Ophtalm Soc 66* 940 1968

16 Robertson B Tunell R & Rudhe U Late stages of pulmonary hyaline membranes of newborn *Acta Paediat Scand 53* 443 1964

17 Rosan R C Porter D Y & Northway W H Jr Pulmonary dysplasia following survival from severe respiratory distress syndrome (RDS) of newborn new disease? *Fed Proc 25* 603 1966

18 Rudhe U & Broberger U Roentgenological observations in survivors of clinical hyaline membrane disease of the newborn *Ann Radiol* (Paris) *10* 289 1967

19 Thibeault W W Grossman H Hagstrom J W C & Auld P A Radiological findings in the lungs of premature infants *J Pediat 74* 1 1969

20 Thomas D V Fletcher G Sunshine P Schafer I A & Klaus M Prolonged respirator use in pulmonary insufficiency of newborn *JAMA 193* 183 1965

21 Wilson M G & Mikity V G New form of respiratory disease in premature infants *Amer J Dis Child* 99 489 1960

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CASE REPORT

HYPERLIPIDEMIA IN TYPE III GLYCOGENOSIS

BOHUMIL VÍTEK DANUŠE ŠRÁČKOVÁ MIROSLAV TOMAN JAROSLAV KRÁTKÝ and JIŘÍ VOGNÁREK

From the Second Paediatric Clinic (Head M Toman) the Clinical Central Laboratory (Head O Teyschl) and the Department of Pathology (Head M Dluhos) Hospital for Sick Children Brno Czechoslovakia

Abnormal glycogenolysis in types I III and VI glycogen storage diseases causes marked changes in the lipid metabolism (7 9 11 15 27 28) Insufficient breakdown of hepatic glycogen to glucose leads to frequent hypoglycaemia where the main source of energy becomes free fatty acids that can be metabolized by various tissues especially the heart and the skeletal muscle When fat mobilization occurs free fatty acids are mainly bound to albumin (24)

This report describes a 4 year observation of a boy suffering from type III glycogenosis associated with hyperlipidemia

CASE REPORT

A K male aged 4 years the third child of healthy nonconsanguineous parents was born after a normal term pregnancy birth weight 3 700 g When 5 months old the first symptoms of the disease appeared There occurred repeated attacks of unconsciousness especially during upper respiratory infections At the same time the abdomen became enlarged The patient was first admitted to our department at the age of 6 months His weight was 6 580 g Medical examination revealed a physically underdeveloped infant There was marked muscular hypotonia and the skin was somewhat pale and without efflorescence The heart was not enlarged there was an insignificant protomesosystolic murmur unsplit second heart sound without accentuation The lung examination was normal the liver was firm and tender and palpable 4 cm below the costal margin and the spleen was not palpable

The diagnosis of a glycogen storage disease was made on the basis of biochemical investigations (2 14) Liver biopsy and later skeletal muscle biopsy Fasting hypoglycaemia was often accompanied by acetonuria The serum and blood glucose response to epinephrine and intramuscular glucagon was minimal after fasting but demonstrable after previous meal (Fig 1) The glucose was determined by the glucose oxidase method The glycogen content of the patient's erythrocytes was 3 164 μg/g Hb the parents 124 μg/g Hb and 146 μg/g Hb (23) Liver biopsy showed the following the hepatocytes were composed of large and pale vacuolated cells The liver cells contained large amounts of PAS-positive material The skeletal muscle (m quadriceps) glycogen content was 6.18 g/100 g of wet tissue (28)

The results of repeated laboratory investigations made on admission and in the 4 year period of observation appear in table Laboratory studies made on admission revealed elevated alpha-globulins (26) SGOT and SGPT (25) The other measured things were without abnormalities

The electrocardiogram and X ray films of the chest revealed no abnormalities

Examination at the age of four revealed a markedly retarded physical development, his height was 89 cm and weight 14 kg The skin was rather pale he had doll like facies with xanthelasma on the upper eyelids Hepatomegaly without cardiomegaly and splenomegaly was present no heart murmur was heard and the second heart and sound was slightly accentuated and closely split There were no abnormalities in the lungs The X ray showed no changes either in the heart or the lungs Blood pressure was 90/60 mm Hg The ECG was normal with sinus rhythm and AQRS + 60 without abnormality Cardiac catheterization was normal except for a slight elevation of right ventricular pressure (40 mm Hg max 6 mm Hg min)

After an intramuscular administration of glucagon blood from the vena hepatica and vena cava superior

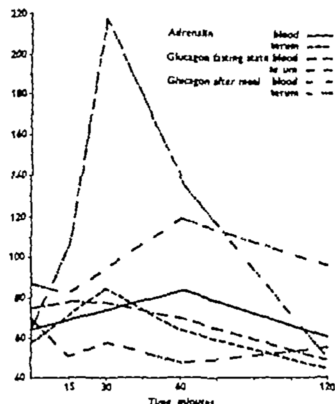


Fig 1 Response of serum and blood glucose to epinephrine and glucagon

was sampled (Fig 2) The administration of glucagon did not increase hepatic glycogenolysis whereas the level of free fatty acids (21) in both the vena hepatica and vena cava superior increased The plasma lactate was found to have remained increased The plasma pyruvate in the hepatic vein was markedly elevated Blood ketone bodies were not measured but there was marked acetonuria

The high levels of total lipids (12) lipoproteins (4) cholesterol (12) and the higher percentage of alpha and beta globulins in the serum electrophoresis (26) indicated marked changes in lipid metabolism (Table 1) The blood glycogen concentration had increased to 38 4 mg/100 ml (25) The structure of glycogen isolated from erythrocytes was determined by iodine complex absorption (16) The curve showed deflections *from the normal course (A)* and had two maximum absorptions (Fig 3) The first occurred at a wave length of 500 mμ the second at a wave length of 410 mμ Glycogen isolated from the erythrocytes of a healthy individual had a maximum iodine absorption at a wave length of 460 mμ It is not easy to interpret the findings The maximum absorption of the glycogen at a wave length of 410 mμ could indicate the presence of limit dextrins whereas the presence of the second maximum absorption at a wave length of 500 mμ could rather indicate the presence of amylopectin Enzyme determination of glycogen was not carried out

DISCUSSION

Recurring hypoglycaemia reflecting defective hepatic glycogenolysis is comon to type I, III and VI glycogenoses Type I glycogenosis is characterized by glucose-6-phosphatase deficiency making release of glucose from glucose 6 phosphate impossible (11) Because of a deficiency of amylo 1 6 glucosidase in type

Table 1 *Biochemical values*

Blood lactate and pyruvate was estimated with lactate dehydrogenase using the reagent sets TC–B TC–C and TC–M (Boehringer Mannheim Germany) The other methods of analysis for the data in the table were described previously (4 12 20 21 25 26)

Age	6 mo	11 mo	13 mo	19 mo	21 mo	4 y
Sedimentation rate	8–12	8–14	—	—	56–70	73–125
Electrophoresis of the serum proteins (in)						
Albumin	55 0	42 0	50 0	—	—	44 2
alpha₁ glob	5 7	4 2	5 0	—	—	7 6
alpha₂ glob	10 3	15 8	8 0	—	—	15 2
beta glob	10 8	15 8	15 5	—	—	7 6
gamma glob	18 2	22 2	21 5	—	—	25 4
Total lipids (mg/100 ml)	320 0	457 0	899 0	630 0	3 380 0	2 220 0
Lipoproteins (mg/100 ml)	520 0	—	—	1 485 0	—	1 730 0
Total cholesterol (mg/100 ml)	*170 0*	*243 0*	*275 0*	*520 0*	—	*958 0*
Serum free fatty acids (μEq/liter)	—	—	—	—	3 6	3 3
Serum bilirubin (mg/100 ml)	0 1	1 5	0 8	.8	0 9	1 2
Thymol turbidity (units)	5 0	12 9	7 5	3 1	6 1	3 6
SGOT (micromol)	8 84	4 32	3 84	3 0	3 5	3 3
SGPT (micromol)	7 31	4 68	3 0	3 2	3 3	3 0
Lactate (mg/100 ml)	—	—	—	26 0	—	51 0
Pyruvate (mg/100 ml)	—	—	—	1 6	—	1 6

III glycogenosis glucose release from glycogen can be effected only from 1 4 glycosidic outer chains In type VI glycogenosis the deficiency of hepatic phosphorylase also prevents a release of sufficient glucose from the outer chains of hepatic glycogen (6 14) The deficiency of amylo-1 6-glucosidase in type III can also be demonstrated in the skeletal and heart muscle the leucocytes and erythrocytes In type I glycogenosis the deficiency of glucose 6-phosphatase has been shown to occur also in the kidneys and the mucous membrane of the small intestine (11)

The main hormonal regulators of glycogenolysis are glucagon and epinephrine Glucagon affects only hepatic glycogenolysis (3 13 18 19) and epinephrine affects the glycogenolysis of the muscle and other tissues as well The effects of glucagon and epinephrine may be due to activation of the phosphorylase system

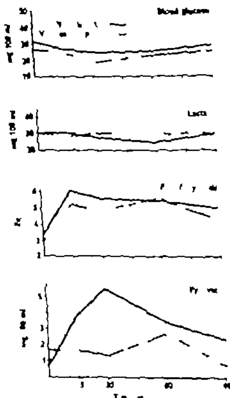


Fig 2 Results of intramuscular administration of glucagon

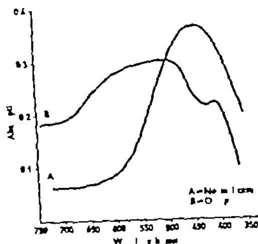


Fig 3 Absorption spectrum of glycogen iodine complex

be increasing the production of adenosine 3 5 cyclic monophosphate through adenyl cyclase activation (3 29) Glucagon epinephrine norepinephrine and cyclic AMP were also found to stimulate gluconeogenesis from lactate and pyruvate in the perfused rat liver (8)

In type III glycogenosis during fasting glucose from the 1 4 glycogen linkages is depleted Further phosphorylase activation by glucagon or epinephrine stimulation cannot therefore cause additional liberation of glucose from phosphorylase limit dextrin therefore no hyperglycaemic response will be observed The hyperglycaemic response to glucagon occurs only after a meal because the synthesis of glycogen is normal in type III glycogenosis (13 18) As the frequency and duration of relative hypoglycaemic intervals increase free fatty acids and perhaps also glycerol from adipose tissues become the main source of energy (1 15 19 22) Hypoglycaemia may be an important factor in the pathogenesis of hyperlipidemia hyperlipoproteinemia and hypercholesterolemia

Elevated free fatty acid levels through enhanced re-esterification of fatty acids could result in an increase of triglyceride synthesis in the liver and subsequent release of lipoproteins into the serum (15) Administration

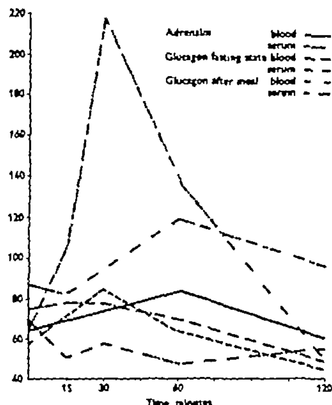


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mination of unesterified fatty acids in blood serum *Physiol Bohemoslov* 10 98 1961
22 Senior B & London L Functional differentiation of glycogenoses of the liver with respect to the use of glycerol *New Eng J Med* 279 965 1968
23 Sidbury J B Cornblath M Fisher J & House E Glycogen in erythrocytes of patients with glycogen storage disease *Pediatrics* 27 103 1961
24 Spector A A The transport and utilization of free fatty acid *Ann NY Acad Sci* 149 768 1968
25 Sevela M To the photometric estimation of serum transaminase comparison of Reitman Frankel method and the electrophoretic method *Vnitrni lek* (Praha) 4 721 1958
26 Tuma A Die Elektrophorese der Blutserum proteine an den Zelluloseacetatstreifen *Publ fac Sci Univ* (Brno) *31* 136 1966
27 Vitek B Hemiplegie u dvou deti s glykogenózou a fibroelastozou endokardu *Cs Pediat* 24 503 1969
28 Vitek B Sracková D Rozprimová L & Snopková J Glykogenóza a její diagnostika *Cs Pediat* 24 567 1969
29 Whitty A J Shima K Trubow M & Foa P P Effect of glucagon and insulin on serum fatty acids in normal and depancreatized dogs *Proc Soc Exp Biol Med* 130 55 1969

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of glucagon effected an increase of free fatty acids in our patient 12 hours postprandial, but did not produce a hyperglycaemic response This can be explained by the increased mobilization of fat from adipose tissues in the liver (29) and perhaps even from peripheral fat by tissue lipase stimulation as has been demonstrated *in vitro* (5)

The influence of glucagon on fat mobilization is difficult to define *in vivo* in most instances since the decrease in free fatty acids observed may be a consequence of the concomitant hyperglycaemia *per se* (17) An elevation of pyruvate in the hepatic vein without an elevation of lactate after glucagon should be very likely originate from glycerol which after hydrolysis changes to triose compounds (Dihydroxyacetone and glyceraldehyde) and joins the main metabolic pathway of carbohydrate (22 24)

SUMMARY

During 4 years of observation, a patient suffering from type III glycogenosis developed considerable hyperlipidemia with hyperlipoproteinemia and hypercholesterolemia The boy repeatedly suffered from hypoglycaemia especially during infections Our diagnosis was made on the basis of markedly increased quantities of glycogen in the liver skeletal muscle and the erythrocytes After a 12-hour fast glucagon administration resulted in an elevation of free fatty acids and pyruvate no change in lactate and no increase in glucose as measured in both the hepatic and peripheral veins These observations can be explained by the influence of glucagon on lipolysis

REFERENCES

1 Bolinger R E Shane S R & Kirkpatrick Ch C Secondary rise in plasma free fatty acids following glucose load *J Clin Endocrin Metabol 22* 873 1962

2 Breton A Gaudier B Ponté C & Lescut J Polycorie glycogénique Contribution des tests cliniques au diagnostic de la forme enzymatique *Pédiatrie* (Lyon) *15* 635 1960

3 Buchanan K D Vance J E Dinstl K Williams R H Hickernell B & Pate S Effect of blood glucose on glucagon in anesthetized dogs *Diabetes 18* 11 1969

4 Burstein M & Samaille J Dosage des beta lipoproteines seriques après precipitation sélective par l héparine *Presse Med 66* 974 1958

5 Camu F Interactions du glucagon de l'insuline et du glucose sur la lipolyse du tissue adipeux de rats in vitro *Arch Int Pharmacodyn 178* 370 1968

6 Cori G T & Larner J Action of amylo-1 6-glucosidase and phosphorylase on glycogen and amylopectin *J Biol Chem 188* 17 1951

7 Creveld S Van & Huijing F Glycogen storage disease Biochemical and clinical data in sixteen cases *Amer J Med 38* 554 1965

8 Exton J H & Park C R Control of gluconeogenesis in liver III Effects of 1 lactate pyruvate fructose glucagon epinephrine and adenosine 3 5 monophosphate on gluconeogenic intermediates in the perfused rat liver *J Biol Chem 244* 1424 1969

9 Fernandes J & Pikaar N A Hyperlipemia in children with liver glycogen disease *Amer J Clin Nutr 22* 617 1969

10 Forbes G B Glycogen storage disease Report of a case with abnormal glycogen structure in liver *J Pediat 42* 645 1953

11 Fuhrmann W Jakovcic S & Hua D Y Y Glycogenose Typ I (v Gierke) fruher mitgeteilt als familiare Hyperlipidemie *Dtsch Med Wschr 91* 453 1966

12 Homolka J *Klinické biochemické vysetrovaci metody* Stát zdrav nakl Praha 1969

13 Hug G Glucagon tolerance test in glycogen storage disease *J Pediat 60* 545 1962

14 Hug G Garancis J C Schubert W K & Kaplan S Glycogen storage disease types II III VIII and IX A biochemical and electron microscopic analysis *Amer J Dis Child 111* 457 1966

15 Jakovcic S Khachadurian A K & Hsia D The hyperlipidemia in glycogen storage disease *J Clin Lab Med 68* 769 1966

16 Krisman C R A method for the colorimetric estimation of glycogen with iodine *Anal Biochem 4* 17 1962

17 Lefebvre P Glucagon et taux sanguin des acides gras non estérifiés chez l homme *Ann Endocrin 26* 602 1965

18 Limbeck G A & Kelley V C Double barrel glucagon test *Amer J Dis Child 109* 162 1965

19 Lowe C U Sokal J E Mosovich L L Sarcione E J & Doray B H Studies in liver glycogen disease Effects of glucagon and other agents on metabolic pattern and clinical status *Amer J Med 33* 4 1962

20 MacLagan N F Liver function tests in the diagnosis of jaundice *Anal Chem 25* 813 1953

21 Novák M An ultramicromethod for the deter

mobilization of unesterified fatty acids in blood serum *Physiol Bohemoslov* 10 98 1961
22 Senior B & Loridan L. Functional differentiation of glycogenoses of the liver with respect to the use of glycerol *New Eng J Med* 279 965 1968
23 Sidbury J B Cornblath M Fisher J & House E Glycogen in erythrocytes of patients with glycogen storage disease *Pediatrics* 27 103 1961
24 Spector A A The transport and utilization of free fatty acid *Ann NY Acad Sci* 149 768 1968
25 Ševela M To the photometric estimation of serum transaminase comparison of Reitman Frankel method and the electrophoretic method *Vnitrní lék* (Praha) 4 721 1958
26 Tuma A Die Elektrophorese der Blutserumproteine an den Zelluloseacetatstreifen *Publ fac Sci Univ* (Brno) 31 136 1964
27 Vlašek B Hemiplegie u dvou deti s glykogenozou a fibroelastózou endokardu *Cs Pediat* 24 503 1969
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CASE REPORT

A CASE OF THE XXXXY CHROMOSOME ANOMALY WITH FOUR MATERNAL X CHROMOSOMES AND DIABETIC GLUCOSE TOLERANCE

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The first case of the chromosome anomaly 49,XXXXY was reported in 1960 by Fraccaro et al (3) In 1966 Zaleski et al (20) reviewed a total of 33 cases Since then at least nine further cases have been reported (1 2 4 5 6 7 10 13 19) Out of these 42 cases 7 were mosaics

Although the clinical picture has some of the features of the syndromes of Down and Klinefelter it forms a separate entity (20)

A new case of the syndrome is described below in a 2 ¹/ year-old boy with a diabetic glucose tolerance test The case is noteworthy too, because it is the fourth case in the literature where the Xg bloodtype investigation in the patient and his parents gives information as to the development of the anomaly

CASE REPORT

The patient was born as the second of twins on July 18 1966 The other twin a girl was stillborn and macerated The parents were unrelated Both were 39 years old at the time of the delivery The father and a 5 year-old sister were healthy and so was the mother before the pregnancy Because of nausea she received Torecan® (thiethylperazini maleas) 6 5 mg perorally 3 times a day during the first 2 months of pregnancy The delivery was medically induced 2 weeks before term because of the following signs in the mother itching slight arterial hypertension proteinuria oedema slight jaundice and bilirubinuria The delivery was uncomplicated His birth weight was 1 900 g and his crown heel length 46 c Immediately after birth he was very hypotonic w insufficient respiration but normal heart action I recovered after suction and oxygen supply Al this the neonatal period was without problems

He was admitted to the University Clinic of Paedi rics Aarhus on the 27th of January 1969 beca of retarded mental and physical development w suspicion of Down s syndrome

On admission he was 81 cm tall which is 11 below the average height for his age (Fig 1) I weight was 11 15 kg The skull was brachycephi and asymmetrical with flattening of the right occip region The ears were low set and slightly malform There were slight hypertelorism and epicanthus a distinct mongoloid slanting of the palpebral fissu The bridge of the nose was broad and flat The I was also characterized by a moderate degree of pr nathism The neck was short with loose and ab dant skin The limbs were hypotonic with poo developed musculature There were bilateral cub valgus and genu valgum and the knee joints co be overextended by about 10 degrees There was four finger crease but the 5th finger was short a radially incurved on both sides The scrotum v hypoplastic and the testes could not be palpated I the penis was normal

There were no signs of organic heart dise Hearing and sight were judged to be normal At ophthalmoscopic examination the papillas w slightly flattened and pale

He was considerably physically retarded He was able to sit on his own but needed support to stand and even when strongly supported he was only able to make awkward walking movements The IQ determined by Cattell s infant intelligence scale was 35

Radiological examination The skull was found scoliotic and brachycephalic The extremities showed delayed bone development especially peripherally (bone age 6 months) Second phalanx of the fifth finger

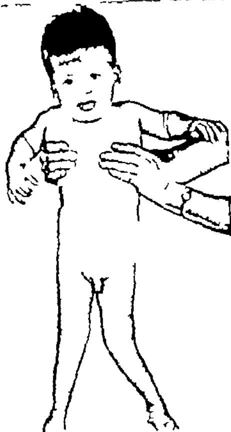


Fig. 1. The patient 31 months old.

and the distal phalanx of the first toe were remarkably short. The dorsal vertebrae were deformed and of reduced height, and there was spina bifida of the fifth lumbar vertebra. No radio-ulnar synostosis was found.

Laboratory investigations. The electroencephalogram during sleep was normal. The serum levels of

Table 1. *Blood sugar and plasma insulin before and after oral administration of 25 g glucose*

Hours	Blood sugar (mg/100 ml)	Plasma insulin (microU/ml)
0	90	7
1/4	57	7
1/2	74	12
3/4	109	15
1	141	28
1½	199	60
2	239	73
2½	245	40
3	156	40

Table 2. *Frequency of 3, 2, 1 and 0 Barr bodies in cells from oral mucosa and cells from cultures of skin fibroblasts*

No. of Barr bodies	3 (%)	2 (%)	1 (%)	0 (%)
Oral mucosa				
1. count	7	29	39	25
2. count	7	32	29	32
Skin				
1. count	27	28	31	14
2. count	30	34	26	10

calcium and thyroxine were normal and so were the seroreactions for toxoplasmosis, cytomegalovirus infection and syphilis. The excretion of acid mucopolysaccharides in the urine was within normal limits. In a 24 hours sample of urine there was an excretion of 45 μMol carnosine, but apart from this the quantitative determination of the amino acids in the urine and plasma was normal (the determination was carried out on a Technicon Amino Acid Analyzer by the Central Laboratory, County Hospital, Aalborg). There was no glucosuria. Blood glucose during fasting was normal, but after oral administration of 25 g glucose a diabetic glucose tolerance was found with a slow and not very great rise in plasma insulin (Table 1) (for method see Nielsen et al. (14)).

Cytogenetical investigations. The sex chromatin was investigated in preparations of oral mucosa stained by the Feulgen method and in cultures of skin fibroblasts grown for 4 days in Leighton tubes and stained with Feulgen light green.

In the preparations from oral mucosa only Barr bodies located at the margin of the nucleus were counted, whereas in the fibroblast cultures Barr bodies with juxtanucleolar and intermediate location were counted as well. 100 nuclei were counted in each of two preparations from each of the two tissues. The results are seen in Table 2.

Counting and analysis of chromosomes were done in 30 metaphases from cultures of peripheral blood by a modification of the method of Moorhead et al. (12) and in 30 metaphases from cultures of a skin biopsy specimen (18) taken from the inside of the upper arm. The distribution of chromosome numbers appears from Table 3. The 3 cells with 48 chromo-

Table 3. *Distribution of chromosome numbers*

	Chromosome count			
	<47	47	48	49
Peripheral blood	0	0	1	29
Skin	0	1	2	27

somes showed E E and C monosomy and the one cell with 47 chromosomes was 47,XXXY with monosomy D

The chromosomes of both parents were investigated in cultures from peripheral blood and both had normal karyotypes

Dermatoglyphic analysis Finger and palms the total ridge count was low 96 (normal average in men about 145) Triradius distance *a–b* was 50 / 50° *b–c* 18 8 /13 3 and *c–d* 31 /37°, the left hand being mentioned first (mean values *a–b* 39 *b–c* 24 *c–d* 37°) The triradius *t* was displaced to *t'* Toes and soles the pattern intensity was low First interdigital interval was large

Xg blood type The father had the type Xg (a+) the patient and his mother Xg (a−)

DISCUSSION

With the increasing use of chromosome investigations it has been shown among other things that different abnormal karyotypes often have clinical symptoms in common Our patient was admitted because of suspicion of Down s syndrome but he appeared to be a typical representative of the XXXXY anomaly

The confusion with Down s syndrome is excusable as the patients with 49,XXXXY are considerably mentally retarded and marked by hypotonia and joint hyperflexibility As a rule they also show mongoloid slanting of the palpebral fissures epicanthus four-finger crease and short incurved fifth finger Other features do to some extent separate these patients from patients with Down s syndrome hypertelorism prognathism and large malformed ears Other frequent findings are coxa valga genu valgum and proximal radio ulnar synostosis Finally the patients show features that may call attention to Klinefelter s syndrome hypogenitalism with underdeveloped scrotum undescended testes and small penis The common finding in testicular biopsy is atrophia of tubuli seminiferi As adults the patients are characterized by eunuchoid proportions and may have gynnecomastia

Often they are born small for dates Their expectation of life is not known The oldest one of the reported cases was 35 years at the time of the investigation (11)

It has been discussed whether the mothers age at the time of the patients birth is higher than normal This age is stated in 36 cases the average being 29 1/2 years This figure should be looked upon with reservation however as probably some of the patients with the XXXXY syndrome born to older mothers are classified as cases of Down s syndrome without chromosome investigation

The chromosome anomaly may be caused by meiotic non disjunction in either egg and/or sperm and eventually by mitotic non-disjunction of the X chromosome in the first divisions of the zygote Investigation of the Xg blood type sometimes makes it possible to decide whether the four X chromosomes in these patients are maternal or paternal In 25 mother father XXXXY combinations so far investigated (17) only 3 have been informative (11 13) and in all 3 cases the distribution was as in our case The patient and his mother were Xg (a−) and the father Xg (a+) Since the Xg^a gene is dominant all the patient s X chromosomes were maternal and the karyotype therefore 49 $X^{M}X^{M}X^{M}X^{M}Y$ Therefore, the most likely explanation of the development of the anomaly is successive non disjunction in the meiotic divisions of the egg (16)

Although glucose tolerance tests have been performed in patients with the XXXXY syndrome (8 9 20) diabetic glucose tolerance has not been reported before in these patients In a way this is surprising considering the increased frequency of diabetes of the maturity onset type in patients with Klinefelter s syndrome (14) The insulin response during peroral glucose tolerance test in these patients is as a rule brisk with the highest values not higher than those found in non diabetics but longer lasting In our patient the response is more like that found in patients with mild juvenile diabetes (14) The explanation of the increased frequency of diabetes in Klinefelter s syndrome is under discussion It is worth noting that a high frequency of diabetes has been found in Turner s syndrome too (15) The genetic unbalance caused by a supernumerary

as well as by a missing X chromosome there fore seems to predispose to diabetes

An increased frequency of diabetes in the mothers of patients with Klinefelter's syndrome has been claimed (14) and some authors have also found an increased frequency of diabetes in the parents of patients with Turner's syndrome (15). If these results are confirmed the most likely explanation is that diabetes mellitus facilitates non-disjunction. There were no known cases of diabetes in our patient's family.

Further investigations are indicated to elucidate these problems, however, and the investigations should also be extended to cases of the XXXXY syndrome to see if there is the same relation between this syndrome and diabetes as between Klinefelter's syndrome and diabetes.

SUMMARY

The 49 XXXXY anomaly was found in a 2½ year-old boy with signs typical of that anomaly: physical and mental retardation, hypogenitalism and certain mongoloid features.

Determination of the Xg blood type in the patient and his parents showed that the X chromosomes were of maternal origin. The most likely explanation of the development of the anomaly is non-disjunction twice during the meiotic divisions of the egg.

The finding of a diabetic glucose tolerance test is discussed in relation to the increased frequency of diabetes mellitus in patients with Klinefelter's syndrome.

ACKNOWLEDGEMENTS

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REFERENCES

1. Flashbart, E., Dehamel, B. & Bardavart, M.: Nouvelle observations du syndrome XXXXY. Ann Pediat 44: 479, 1968.
2. Ferrante, R., Bruni, L., Vignetti, P. & Valeri, O. M.: Sindrome Klinefelter con cariotipo XXXXY in un bambino di 3 anni. Arch Ital Pediat 24: 275, 1966.
3. Fraccaro, M., Kaijser, K. & Lindsten, J.: A child with 49 chromosomes. Lancet II: 899, 1960.
4. Frøland, A.: Klinefelter's Syndrome. Thesis. Copenhagen 1969.
5. Galindo, J. & Barr, H. S.: The XXXXY sex chromosome abnormality. Arch Dis Childh 41: 82, 1966.
6. Gedikoglu, A. O.: Cok nadir bir Klinefelter sendromu XXXXY kromozom konstitusyonu. Tip Fak Mec (Istanbul) 30: 236, 1967.
7. Grouchy, J. de, Frezal, J., Bitan, A. & Lamy, M.: Un cas de mosaïque XXXXY/XXXY. Ann Genet 8: 63, 1965.
8. Jancar, J.: Mentally defective males with XXXXY chromosomes. In J. Oster & H. V. Sletved (eds.): Paper presented at international congress on the scientific study of mental retardation, Copenhagen 1963, p. 179.
9. Lamy, M., Aussannaire, M., Grouchy, J. de & Lalande, J.: Mosaïque XXXXY/XXXY dans le syndrome de Klinefelter. Ann Pediat 39: 167, 1963.
10. Lauritsen, J., Frøland, A. & Johnsen, S. G.: Sexual differentiation in the XXXXY chromosome constitution. Acta Path Microbiol Scand 65: 321, 1965.
11. Lewis, F. J. W., Frøland, A., Sanger, R. & Race, R. R.: Source of the X chromosomes in two XXXXY males. Lancet II: 589, 1964.
12. Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M. & Hungerford, D. A.: Chromosome preparations of leucocytes cultured from human peripheral blood. Exp Cell Res 20: 613, 1960.
13. Merten, J. D. & Scholz, W.: Serologische Klärung der Herkunft der überzähligen X-Chromosomen beim XXXXY-Syndrom. Blut 16: 164, 1967.
14. Nielsen, J., Johansen, K. & Yde, H.: Frequency of diabetes mellitus in patients with Klinefelter's syndrome of different chromosome constitutions and the XYY syndrome. Plasma insulin and growth hormone level after a glucose load. J Clin Endocr 9: 1062, 1969.
15. — The frequency of diabetes mellitus in patients with Turner's syndrome and pure gonadal dysgenesis. Acta Endocr 62: 251, 1969.
16. Race, R. R. & Sanger, Ruth: Xg and sex-chromosome abnormalities. Brit Med Bull 25: 99, 1969.
17. Sanger, R.: Personal communication.
18. Therkelsen, A. J.: Sandwich technique for the establishment of cultures of human skin for chromosome investigation. Acta Path Microbiol Scand 61: 317, 1964.

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CASE REPORT

PROLONGED SURVIVAL IN NEUROBLASTOMA WITH MULTIPLE SKELETAL METASTASES AND BONE MARROW INFILTRATION

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Review of the literature has revealed only two recorded children with disseminated skeletal metastases and bone marrow infiltration secondary to neuroblastoma who have survived for longer than 5 years In view of the paucity of reported cases of neuroblastoma with osseous and bone marrow metastases who have had long term survival we report an 8 year 2 month old female who is well without evidence of disease 7 years after having had neuroblastoma of the left adrenal gland accompanied by radiographic evidence of multiple bone metastases and histologic evidence of bone marrow infiltration by neuroblastoma

CASE REPORT

In October 196 a 14 month-old Caucasian girl the product of a full term normal gestation and delivery was admitted to New York Hospital and on physical examination an upper quadrant abdominal mass was palpated Intravenous pyelogram showed a suprarenal mass containing calcium and displacing the left upper collecting system downward Urinary vanillylmandelic acid excretion was 31 3 mg/24 hours (normal adult range 0 1 9 0 mg 24 hours) Radiographic skeletal survey revealed an irregular lytic defect in the distal metaphysis of the left tibia with periosteal reaction irregularity of the frontal bone and lytic lesions in both orbital wings both femoral necks and right fifth rib (Figs 1 and 3) Biopsy of the left tibia disclosed infiltration of the bone marrow by nests of darkly staining tumor cells arranged in small nodules

The patient was treated with irradiation (2 000 r and 1 500 r respectively) to the primary tumor of the left adrenal gland and to the left tibia followed by cyclophosphamide and methotrexate Cyclophosphamide was administered in a dose of 15 mg per kg body weight intravenously followed by 5 0 mg per kg daily orally for 2 months and for a further month in a dose of 3 0 mg per kg on alternate days Methotrexate was administered orally in a dose of 1 25 mg daily for 2 months and in the same dosage on alternate days for a period of 21 months She had frequent radiographic skeletal surveys and in May 1964 for the first time she displayed no roentgenologic evidence of metastatic disease In October 1964 24 hour urinary norepinephrine level was 24 38 μg epinephrine 5 63 μg and vanillylmandelic acid 113 μg All these values are within normal limits In December 1964 the primary tumor and entire left adrenal gland were removed and the left mesenteric and para-aortic nodes resected The resected tissue did not reveal any tumor cells

In May 1967 the patient presented with signs of small bowel obstruction Exploratory laparotomy was carried out and adhesions obstructing the small bowel were resected No abdominal tumor was present at that time and repeat vanillylmandelic acid excretion and roentgenological skeletal survey were normal The girl was last seen in the New York Hospital Pediatric Hematology Clinic in April 1970 and she continues to be well without any evidence of neuroblastoma clinically chemically or radiographically

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DISCUSSION

Neuroblastoma is a disease with a variable clinical course ranging from prolonged remis-

19 Tsenghi C Metaxotou A Economou-Mavrou K & Matsaniotis N An XXXXY male *Delt Paidat Klin Panep Athin 13* 487 1966
20 Zaleski W A Houston C S Pozsonyi J & Ying K L The XXXXY chromosome anomaly report of three new cases and review of 30 cases from the literature *Canad Med Ass J 94* 1143 1966

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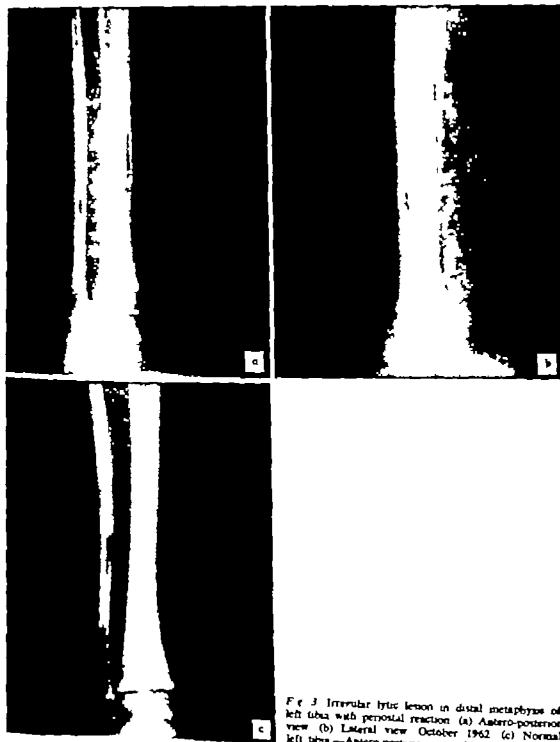


Fig. 3. Irregular lytic lesion in distal metaphysis of left tibia with periosteal reaction. (a) Antero-posterior view. (b) Lateral view. October 1962. (c) Normal left tibia.—Antero-posterior view. May 1964.

tion to rapidly disseminating disease and death. Various factors have been considered useful in determining prognosis in this disease. These factors include age of patient at time of diagnosis, extent of disease, tumor cytology, site of primary, and therapy employed (2–4). An



Fig 1 Irregular lytic lesion of frontal bone



Fig 2 Lytic lesions of both is chial wings and femoral necks

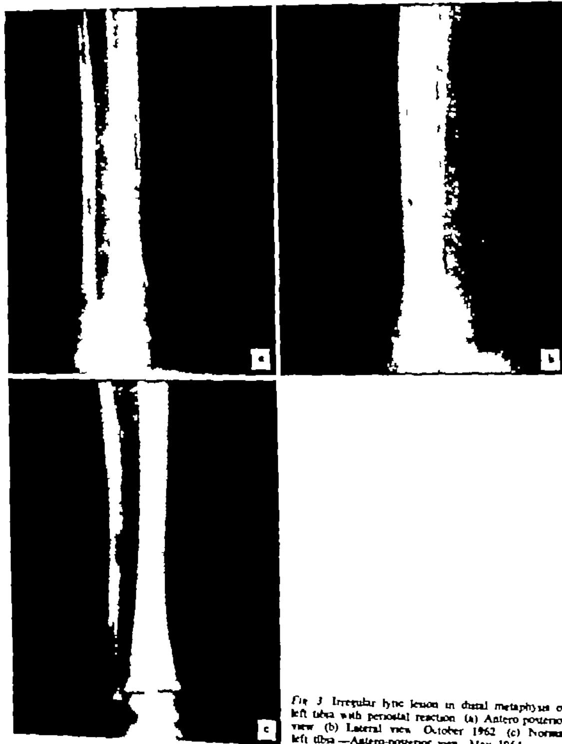


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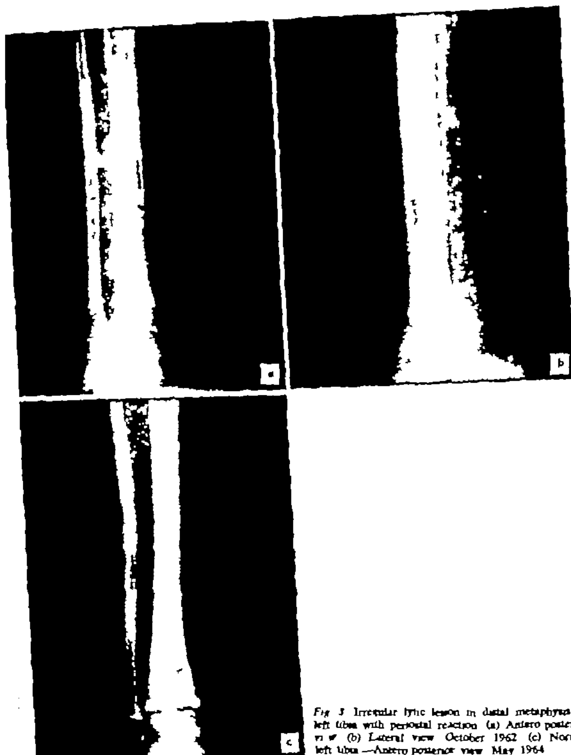


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especially poor prognosis has been associated with skeletal metastases and/or bone marrow infiltration

With reference to skeletal metastases, Fortner et al (2) reported survival for longer than 5 years in four patients with bony metastases in neuroblastoma in a series of 133 patients but none of their patients with bone marrow infiltration survived for 5 years Reilly et al (4) reported long term survival of three children with disseminated skeletal metastases secondary to neuroblastoma and in review of the literature they found only eight additional patients who had survived longer than 2 years after the development of metastatic bone lesions

Bodian (1) and James et al (3) have reported prolonged survival following bone marrow infiltration in neuroblastoma in which there has been no demonstrable roentgenologic evidence of skeletal dissemination Bodian (1) reported a male infant who presented at 4 months of age with several subcutaneous nodules histologically characteristic of neuroblastoma and with tumor cells present in the bone marrow but no radiologic evidence of osseous dissemination demonstrable The patient was seen 4 years and 7 months following diagnosis and physical examination bone marrow examination and urinary vanillyl mandelic acid were normal James et al (3) reported a 5 week old female infant who presented with metastatic neuroblastoma to bone marrow and lymph nodes The site of primary tumor remained undetermined an she was given a 12 month course of vincristine and cyclophosphamide with complete remission for 2 years

There are only two patients on record who have had both skeletal metastases and bone marrow infiltration in neuroblastoma who have had prolonged survival and both these cases shared the relatively favorable prognostic signs of an extra adrenal primary neoplasm and clinical onset of disease during the first year of life (4) The patient reported in this paper had a tumor which was adrenal in origin and at the time of diagnosis was 14 months of age

SUMMARY

The present report is the third recorded case, to our knowledge, of a child with multiple skeletal metastases and bone marrow infiltration secondary to disseminated neuroblastoma who has survived 7 years and 6 months after the diagnosis without clinical chemical or radiographic evidence of disease

REFERENCES

1 Bodian M Neuroblastoma An evaluation of its natural history and the effects of therapy with particular reference to treatment by massive doses of vitamin B *Arch Dis Child 38* 606 1963
2 Fortner J Nicastri A & Murphy M L Neuroblastoma Natural history and results of treatment in 133 cases *Ann Surg 167* 132 1968
3 James D H Hustu O Wrenn E C & Pinkel D Combination chemotherapy of childhood neuroblastoma *JAMA 194* 123 1965
4 Reilly D Nesbit M E & Krivit W Cure of three patients who had skeletal metastases in disseminated neuroblastoma *Pediatrics* 41 47 1968

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Key words Neuroblastoma skeletal metastases bone marrow infiltration

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CASE REPORT

FAMILIAL EARLY HYPOPARATHYROIDISM ASSOCIATED WITH HYPOMAGNESAEMIA

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Idiopathic hypoparathyroidism depends on a deficiency of parathyroid hormone and is considered to be a relatively rare disease. Patients suffering from idiopathic hypoparathyroidism must fulfil the following criteria 1) low serum calcium level 2) high serum inorganic phosphorus level 3) absence of radiological signs of rickets and osteomalacia 4) chronic tetany 5) absence of renal insufficiency steatorrhea chronic diarrhea and alkalosis (8) They should present acute phosphate diuresis following the intravenous administration of parathyroid hormone (29)

It is the purpose of this paper to present two cases of idiopathic hypoparathyroidism occurring in siblings diagnosed during their first year of life with evidence of non sex linked heredity and a low serum magnesium level

CASE REPORTS

Case 1

S F Girl born at full term in breech presentation She gained weight poorly and her development was slow At the age of 3 months she had a short attack of convulsions A relapse occurred at 5 1/2 months when she was first seen at the hospital There were no signs of rickets Her serum calcium was 2.25 mEq/l and serum inorganic phosphate 10.0 mg/100 ml EEG showed paroxysmal dysrythmia but it has since changed to normal She had not received the usual vitamin D prophylaxis and was now given 500 000 U vitamin D resulting in normal serum calcium and inorganic phosphorus levels The patient was first thought to have spasmophilia For later data about serum calcium level and therapy see Fig 1

At the age of 12 months she had tetany related to an infection with moderate elevation of temperature She had no teeth and Chvostek's sign was positive Idiopathic hypoparathyroidism was suspected and the patient was referred to the Department of Paediatrics at Kronprinsessan Lovisas Barnsjukhus Karolinska Institutet Stockholm where the diagnosis was verified For further information see Aperia et al case S F (1)

At 20 months of age she was found to have enamel hypoplasia Bilateral cataracts were not observed before the age of 4 years and 10 months She had no radiological signs of rickets intracranial or intrarenal calcium deposits She had an IQ of 75 (Terman Merril scale) and signs of emotional lability

Great variations of the serum calcium level occurred and it has been difficult to balance therapy accordingly At periods when the serum calcium level was high she was tired lost her appetite and was irritable

Case 2

A F Sister of S F born at full term with normal delivery She was immediately transferred to the department of paediatrics in order to follow the serum levels of calcium and inorganic phosphorus (Fig 2) Chvostek's sign was positive at low serum calcium levels Like her sister she was tired irritable and lost her appetite when hypercalcaemia was present and balanced therapy was difficult to achieve Signs of tetany only occurred when the patient had hypocalcaemia in connection with fever due to infections There were no signs of rickets EEG (when she had normal serum calcium level) X ray of the skull and eye examinations were normal Normal eruption of the teeth occurred but there was enamel hypoplasia at the age of 1 year and 10 months The Ellsworth-Howard test was performed at the age of 1 year and 11 months and was positive During the

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This case is earlier described as case S F by Aperia et al in 1967 (1)

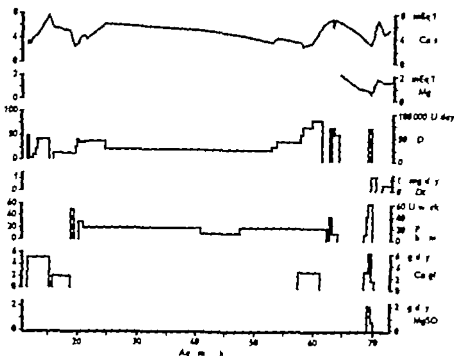


Fig 1 Case 1 S F Survey of changes in serum calcium and serum magnesium in relation to therapy D vitamin D Dt dihydrotachysterol Ca gluc calciumgluconate

test there were no clearcut changes of the blood chemistry Serum magnesium rose from 1 1 to 1 4 mEq/l In the control case serum magnesium increased from 1 3 to 1 5 mEq/l

Both case have had hypomagnesaemia with lowest serum levels at 0 5 and 0 4 mEq l respectively determined with the fluorimetric method described by Schacher in 1962 (26) After institution of oral 7 magnesium sulphate solution the serum level of magnesium became normal see Figs 1 and 2

They have not had moniliasis and have not shown any evidence of renal dysfunction malabsorption or associated endocrine disorders

FAMILY HISTORY

Convulsions were common in members of this family where the parents are first cousins see pedigree (Fig 3) and Table 1 The grandmother of S F and A F (Fig 3 II 2) has an abnormal EEG probably due to trauma The great grandmother and one of her sisters were said to suffer from epilepsy

Of the mother's siblings five are dead only one (Fig 3 III 4) was hospitalized This girl was born at full term in a normal delivery At the age of 4 weeks she was fed with cow's milk Diarrhoea resulted and 2 days later convulsions sometimes associated with cyanosis occurred These symptoms continued until she died of bronchopneumonia at the age of 2 months The serum calcium level was never determined Autopsy revealed numerous glious cells with fatty globules scattered in the white matter of the cerebrum and in the mesencephalon

For further information about the relatives see pedigree (Fig 3) and Tables 1 and 2

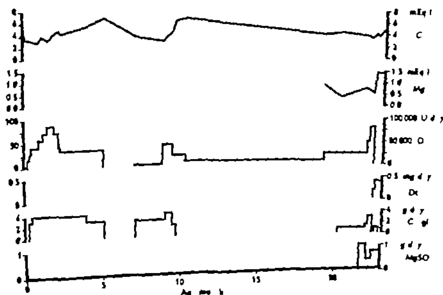


Fig 2 Case 2 A F Survey of changes in serum calcium and serum magnesium in relation to therapy D vitamin D Dt dihydrotachysterol Ca gluc calciumgluconate

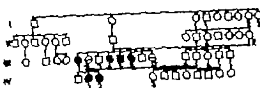


Fig 3 Pedigree □ boys ○ girls [○ dead after a period of convulsions ◑ minor convulsions in infancy ● reported cases

DISCUSSION

The two sisters S F and A F fulfil the criteria of idiopathic hypoparathyroidism This diagnosis cannot b proved for their mother's siblings but their symptoms are well in accordance with those of idiopathic hypoparathyroidism and in one case the cerebral changes resembles those of earlier described cases (10 16)

Idiopathic hypoparathyroidism is very rare during the first year of life Bronsky et al in 1958 collected 50 cases of idiopathic hypoparathyroidism three of which were younger than 15 months of age (5) In literature 23 cases with hypocalcaemic convulsions due to idiopathic hypoparathyroidism at an age less than one year (early onset) are found (Table 3) Six girls 16 boys and one patient of unknown sex are reported There are a few more cases described where the diagnosis is not quite clear (11 13) and the disease may be more common than actually reported In 1967 Geertinger (12) reported a high frequency of parathyroid anomalies in cases of sudden unexpected death in infancy

Table 1 *Individuals in the pedigree (Fig 3) dead at an early age*

No	Age at death	Cause
I 1	9 years	Unknown
I 2	6 months	Morbilli
I 3	4 weeks	Sudden unexplained death
II 1	15 months	HCl-intoxication
III 1	A few months	Convulsions
III 3	8 days	Immaturity
III 4	2 months	Convulsions
III 5	5½ months	Convulsions
III 6	3½ months	Convulsions

Table 2 *Blood chemistry of relatives of cases 1 and 2 Ca and Mg (mEq/l) P (mg/100 ml)*

For numbers see pedigree (Fig 3)

No	Serum levels of Ca	P	Mg
II 2	4 8	3 4	1 6
III 2	4 9	2 6	1 7
III 7	4 1	2 4	1 6
IV 3	5 1	3 1	1 5

Hereditary factors have been found in cases of late onset associated with moniliasis or Addison's disease (9 19 22 30) Of cases with early onset (Table 3) three boys are siblings

Table 3 *Known cases of the early type of idiopathic hypoparathyroidism*

No = reference number T = thymus P = parathyroid glands a = aplasia h = hypoplasia

No	Year of publ	Sex	Age at first symptom	T	P	Age at death	Anomalies of other organs
4	1927	♂	4 months	h	h	4 months	Spinal cord
25	1937	♂	6 weeks	h	h	10 weeks	Thyroid gland
15	1932		9 hours		a	9 hours	Multiple
24	1938	♂		h		12 weeks	Urinary tract thyroid gland
3	1955	?	8 weeks		a	5 months	Cerebrum
6	1955	♂	7 days				
6	1955	♂	9 days				
23	1956	♀	11 days				Glaucoma
7	1957	♂	3 weeks				
28	1957	♂	2 week				
2	1958	♂	Less than 1 year				
17	1959	♂	12 hours	a	a	55 days	
31	1959	♂	A few weeks				
31	1959	♂	35 days				
10	1960	♀	5 days	h	h	11 days	Heart gall bladder
21	1960	♂	7 weeks				
21	1960	♂	5 days				
34	1962		40 days		h	3 months	
16	1963	♂	27 days				Urinary tract
16	1963	♂	3 weeks				
26	1963	♂	1 week			2½ weeks	
32	1965	♀	2 hours	a	a	4 weeks	
15	1967	♂	Newborn	a	a	6 months	Heart
Own case		♀	3 months				
Own case		♀	2 days				

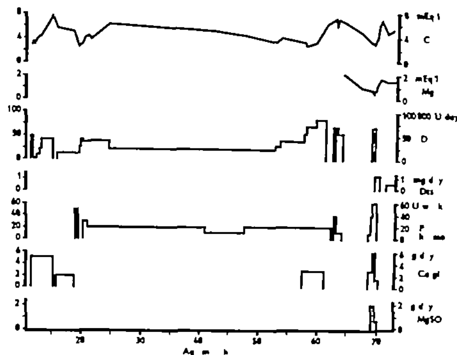


Fig 1 Case 1 S F Survey of changes in serum calcium and serum magnesium in relation to therapy D vitamin D Dts dihydrotachysterol Ca gluc calciumgluconate

test there were no clearcut changes of the blood chemistry Serum magnesium rose from 1 1 to 1 4 mEq/l In the control case serum magnesium increased from 1 3 to 1 5 mEq/l

Both case have had hypomagnesemia with lowest serum levels at 0 5 and 0 4 mEq/l respectively determined with the fluorimetric method described by Schacher in 1962 (26) After institution of oral 7 magnesium sulphate solution the serum level of magnesium became normal see Figs 1 and 2

They have not had moniliasis and have not shown any evidence of renal dysfunction malabsorption or associated endocrine disorders

FAMILY HISTORY

Convulsions were common in members of this family where the parents are first cousins see pedigree (Fig 3) and Table 1 The grandmother of S F and A F (Fig 3 II 2) has an abnormal EEG probably due to trauma The great grandmother and one of her sisters were said to suffer from epilepsy

Of the mother's siblings five are dead only one (Fig 3 III 4) was hospitalized This girl was born at full term in a normal delivery At the age of 4 weeks she was fed with cow's milk Diarrhoea resulted and 2 days later convulsions sometimes associated with cyanosis occurred These symptoms continued until she died of bronchopneumonia at the age of 2 months The serum calcium level was never determined Autopsy revealed numerous glious cells with fatty globules scattered in the white matter of the cerebrum and in the mesencephalon

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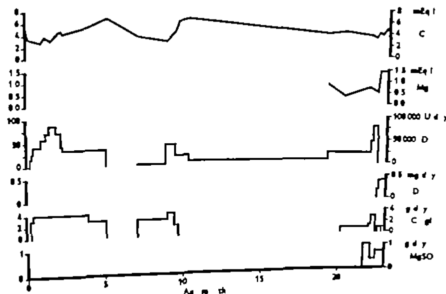


Fig 2 Case 2 A F Survey of changes in serum calcium and serum magnesium in relation to therapy D vitamin D Dts dihydrotachysterol Ca gluc calciumgluconate

17 Lobdell D H Congenital absence of the parathyroid glands *Arch Path* 67 412 1959

18 MacIntyre I Boss S & Troughton V A Parathyroid hormone and magnesium homeostasis *Nature* 198 1058 1963

19 Morse W I Cochrane W A & Landrigan P L Familial hypoparathyroidism with pernicious anemia steatorrhea and adrenocortical insufficiency (A variant of mucoviscidosis) *New Eng J Med* 264 1021 1961

20 Paunier L Radde I C Kooh S W Conen P E & Fraser D Primary hypomagnesemia with secondary hypocalcemia in an infant *Pediatrics* 41 385 1968

21 Peden V H True idiopathic hypoparathyroidism as a sex linked recessive trait *Amer J Hum Genet* 12 323 1960

22 Quinto M G Leikin S L & Hung W Pernicious anemia in a young girl associated with idiopathic hypoparathyroidism familial Addison's disease and moniliasis *J Pediat* 64 241 1964

23 Rhyne J L & Carriker F R Idiopathic hypoparathyroidism and bilateral congenital glaucoma in the neonatal period *Pediatrics* 18 448 1956

24 Rössle R Über den angeborenen Mangel der Epithelkörperchen *Schweiz Med Wschr* 29 848 1938

25 — Über gleichzeitige Missbildungen der branchiogenen Organe und über angeborenen Mangel der Epithelkörperchen *Virchows Arch Path Anat* 283 41 1932

26 Schachter D The fluorometric estimation of magnesium in serum and in urine *J Lab Clin Med* 54 763 1959

27 Smith F G & Zike K Idiopathic hypoparathyroidism in the neonatal period *Amer J Dis Child* 105 182 1963

28 Soyer R T Mason E E & Buckwalter J A Hypoparathyroidism in children and adolescents *Pediatrics* 20 1097 1957

29 Strom L & Winberg J Idiopathic hypoparathyroidism *Acta Paediat Scand* 43 574 1954

30 Sutphin A Albright F & McCune D J Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moniliasis *J Clin Endocr* 3 625 1943

31 Symon L & Bett I M Idiopathic hypoparathyroidism with normal urinary calcium *Brit Med J* 5135 1451 1959

32 Taitz L S Zarate Salvador C & Schwartz E Congenital absence of the parathyroid and thymus glands in an infant (III and IV pharyngeal pouch syndrome) *Pediatrics* 38 412 1966

33 Vague G Giraud R Bernard R Miller G Bernard P Torresani J & Louchet E Hypoparathyroidie grave de la premiere enfance Evolution clinique et biochimique *Ann Endocr* 0 312 1959

34 Yoon Z Garty R Casper J & Benbassat M Primary hypoparathyroidism in an infant Hypoplastic aberrant parathyroid glands in the pharyngeal submucosa *Amer J Dis Child* 104 307 1962

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(6 7) Convulsions in infancy of relatives have been reported (33) These cases could belong to a group classified by Peden in 1960 as idiopathic hypoparathyroidism with early onset inherited as a sex linked recessive trait (21) The present cases cannot belong to this group which consists of boys only Another group of cases is described with early onset of idiopathic hypoparathyroidism This group consists of sporadic cases often with multiple congenital anomalies (21) The author s cases do not belong to this group either Most probably their disease is inherited as a non sex linked trait It could be an intermediate trait with only minor symptoms in the heterozygote cases However then one would expect more members of the family to have symptoms

Hypomagnesaemia associated with the early type of idiopathic hypoparathyroidism has been described in one case (32) Hypomagnesaemia associated with low serum calcium but normal serum inorganic phosphorus levels has also been reported Therapy with calcium and parathyroid hormone was ineffective in this case (20)

In rats the urinary excretion of calcium and magnesium is decreased at the same time as the phosphate excretion is increased after administration of parathyroid hormone (18) Magnesium deficient rats develop hypercalcaemia and hypophosphataemia and the serum magnesium level is decreased by total parathyroidectomy (14) According to this one would expect hypomagnesaemia to be a sign of idiopathic hypoparathyroidism This is confirmed by the previous case studied (32) and by the cases of this report The result of the Ellsworth Howard test in case 2 in contrast to the previous case (32) seems to confirm the effect of parathyroid hormone found in experiments on rats

SUMMARY

Two cases of idiopathic hypoparathyroidism are reported and previous cases of the disease with symptoms at an age less than one year are briefly reviewed Unlike earlier findings there is evidence of the disease as a non sex linked trait, probably recessive Both cases had hypomagnesaemia Administration of parathyroid hormone caused in one patient a rise of the serum magnesium level which has previously been observed in experiments on the rat

REFERENCES

1 Aperia A Broberger O Bergstrand C G & Zetterstrom R Renal response to intravenous phosphate load in idiopathic hypoparathyroidism and pseudohypoparathyroidism *Acta Paediat Scand 56* 357 1967

2 Bergstrand C G Ekengren K Filipsson R & Hugeert A Pseudohypoparathyroidism Familial incidence and comparison with idiopathic hypoparathyroidism *Acta Endocr 29* 201 1958

3 Blaim A & Lewicki Z Zespol tezyczkowy oraz ogolny niedorozwoj u niemowlecia z wrodzonym brakiem przytarczyc *Pediat Pol 30* 923 1955

4 Bottiger E & Wernstedt W Beitrage zur Kenntnis der spasmophilen Diathese IV Mitteilung Todlich verlaufender Fall von Spasmophilie bei einem Brustkinde mit Anomalien der Thymus und der Parathyreoideae *Acta Paediat Scand 6* 373 1927

5 Bronsky D Kushner D S Dubin A & Snapper I Idiopathic hypoparathyroidism and pseudohypoparathyroidism Case report and review of the literature *Medicine 37* 317 1958

6 Buchs S Familiarer Hypoparathyreoidismus *Ann Pediat 184* 364 1955

7 — Familiarer Hypoparathyreoidismus *Ann Pediat 188* 124 1957

8 Drake T G Albright F Bauer W & Castleman B Chronic idiopathic hypoparathyroidism Report of six cases with autopsy findings in one *Ann Intern Med 12* 1 751 1939

9 Edgren G Fall av idiopatisk tetani *Lakartidningen 50* 1 722 1953

10 Farber S & Wawter G F Clinical pathological conference *J Pediat 56* 690 1960

11 Forbes G B Clinical features of idiopathic hypoparathyroidism in children *Ann N Y Acad Sci 64* 432 1956

12 Geertinger P Sudden unexpected death in infancy With special reference to the parathyroids *Pediatrics 39* 43 1967

13 Harrison H E Idiopathic hypoparathyroidism *Pediatrics 17* 442 1956

14 Heaton F W The parathyroid glands and magnesium metabolism in the rat *Clin Sci 28* 543 1965

15 Huber J Cholnoky P & Zoethout H E Congenital aplasia of parathyroid glands and thymus *Arch Dis Child 42* 190 1967

16 Kunstadter R H Tanman W O F & Cornblath M Idiopathic hypoparathyroidism in the newborn *Amer J Dis Child 105* 499 1963

17 Lobdell D H Congenital absence of the parathyroid glands *Arch Path* 67 412 1959
18 MacIntyre I Boss S & Troughton V A Parathyroid hormone and magnesium homeostasis *Nature* 198 1 058 1963
19 Morse W I Cochrane W A & Landrigan P L Familial hypoparathyroidism with pernicious anemia steatorrhea and adrenocortical insufficiency (A variant of mucoviscidosis) *New Eng J Med* 264 1 021 1961
20 Paunier L Radde I C Kooh S W Conen P E & Fraser D Primary hypomagnesemia with secondary hypocalcemia in an infant *Pediatrics* 41 385 1968
21 Peden V H True idiopathic hypoparathyroidism as a sex linked recessive trait *Amer J Hum Genet* 12 323 1960
22 Quinto M C Leikin S L & Hung W Pernicious anemia in a young girl associated with idiopathic hypoparathyroidism familial Addison's disease and moniliasis *J Pediat* 64 241 1964
23 Rhyne J L & Carriker F R Idiopathic hypoparathyroidism and bilateral congenital glaucoma in the neonatal period *Pediatrics* 18 448 1956
24 Rössle R Über den angeborenen Mangel der Epithelkörperchen *Schweiz Med Wschr* 29 848 1938
25 — Über gleichzeitige Missbildungen der branchiogenen Organe und über angeborenen Mangel der Epithelkörperchen *Virchow Arch Path Anat* 285 41 1932
26 Schachter D The fluorometric estimation of magnesium in serum and in urine *J Lab Clin Med* 54 763 1959
27 Smith F G & Zike K Idiopathic hypoparathyroidism in the neonatal period *Amer J Dis Child* 105 182 1963
28 Soper R T Mason E E & Bockwalter J A Hypoparathyroidism in children and adolescents *Pediatrics* 20 1 097 1957
29 Strom L & Winberg J Idiopathic hypoparathyroidism *Acta Paediat Scand* 43 574 1954
30 Sutphin A Albright F & McCune D J Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moniliasis *J Clin Endocr* 3 625 1943
31 Symon L & Bett I M Idiopathic hypoparathyroidism with normal urinary calcium *Brit Med J* 5135 1 451 1959
32 Taitz, L S Zarate Salvador C & Schwartz E Congenital absence of the parathyroid and thymus glands in an infant (III and IV pharyngeal pouch syndrome) *Pediatrics* 38 412 1966
33 Vague G Giraud R Bernard R Miller G Bernard P Torresani J & Louchet E Hypoparathyroidie grave de la première enfance Evolution clinique et biochimique *Ann Endocr* 20 312 1959
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NEW BOOKS RECEIVED

M Adinolfi (ed) *Immunology and development* Clinics in Developmental Medicine no 34 185 pp illus W Heinemann Medical Books Ltd London 1969 63s

W E Nelson V C Vaughan III & R J McKay (eds) *Textbook of Pediatrics* 1590 pp 527 illus W B Saunders Co Ltd Philadelphia London and Toronto 1969 £9 3s

R J Robinson (ed) *Brain and early behaviour* 374 pp illus Academic Press Inc London 1970 100s

M Joseph & C Dawbarn *Measurement of the Facies A study in Down's syndrome* 114 pp illus S I M P Research Monograph No 3 W Heinemann Medical Books Ltd London 1970 50s

G Joppich & H Wolf (eds) *Metabolism of the new born child* 448 pp illus Hippokrates Verlag Stuttgart 1969 DM 75

N R Finnie *Born med cerebral parese* 192 pp illus Borgens Forlag Kopenhamn 1970 D Kr 36 00

Y Manuel J P Revillard & H Beteul (eds) *Proteins in normal and pathological urine* 354 pp illus S Karger Basel 1969 DM 90

L Tuft & H L Mueller *Allergy in children* 561 pp illus W B Saunders Co Ltd Philadelphia London and Toronto 1970 £8 6s

P G Jones (ed) *Clinical paediatric surgery Diagnosis and management* 528 pp illus J Wright & Sons Ltd Bristol 1970 120s

Report of a WHO Scientific Group *Genetic factors in congenital malformations* 42 pp World Health Organization Technical Report Series 1970 no 438 US $1 00

A Ambrose (ed) *Stimulation in early infancy* 289 pp Academic Press London and New York 1970 £4 00

P C Arquembourg J E Salvaggio & J N Bickers *Primer of immunoelectrophoresis* With interpretation of pathologic human serum pattern 84 pp 117 illus S Karger AG Basel München Paris and New York 1970 DM 33

W R Aykroyd *Conquest of deficiency diseases Achievements and prospects* 98 pp World Health Organization Geneva 1970 US $3 00

G Debrun & C Gasquet *Explorations vasculaires des tumeurs abdominales de l'enfant* Monographies des Annales de Radiologie 4 92 pp 44 illus Expansion Scientifique Française P ris 1970 F 40

S S Gellis & B M Kagan (eds) *Current pediatric therapy—4* 1077 pp W B Saunders Co Ltd London 1970 £11 9s 6d

O Grosser & R Ortmann *Grundriss der Entwicklungsgeschichte des Menschen* 207 pp 200 illus Springer Verlag Berlin Heidelberg and New York 1970 DM 28

R Frey & Th Baumann (eds) *Nephrologie im Kindesalter II* Pädiatrische Fortbildungskurse für die Praxis 114 pp illus S Karger AG Basel München Paris and New York 1970 US $7 20

W A Fuchs J W Davidson & H W Fischer *Recent results in cancer research* Vol 23 *Lymphographie in cancer* 294 pp 189 illus Springer Verlag Berlin Heidelberg and New York 1969 DM 76

H Salem & D M Aviado (eds) *Antitussive agents* Vol I II III International Encyclopedia of Pharmacology and Therapeutics Section 27 Pergamon Press Oxford New York and Toronto 1970 £12 US $32 00

J Huntingford K A Huter & E Saling (eds) *Perinatal medicine* 1st European Congress Berlin 299 pp 197 illus Georg Thieme Verlag Stuttgart 1970 DM 37

S Jakowska (ed) *Cystic fibrosis and related human and animal diseases* 240 pp Gordon and Breach Science Publ Inc New York 1970 US $19 50

P Kepler *Grundlagen und Fortschritte der Leukämiebehandlung beim Kinde* 158 pp illus Ferdinand Enke Verlag Stuttgart 1970 DM 36

F S Perkins (ed) *Recent research on the retina* 178 pp British Medical Bulletin Vol 26 No 2 London 1970 £2

E Ransemar & H Renling *Det idealiska sommarnet let En studie hur neurosedyntragedin publicerades* 112 pp Raben & Sjogren Stockholm 1970 Sw kr 9 30

G Rooth *Clinical acid base and electrolyte balance* 80 pp Studentlitteratur Lund 1970 Sw kr 14 30

H G Schwarzacher & U Wolf (ed) *Methoden in der medizinischen Cytogenetik* 186 pp illus Springer Verlag Berlin Heidelberg and New York 1970 DM 48

K Tóth *The epidemiology of dental caries in Hungary* 283 pp Akadémiai Kiadó Budapest 1970 US $10 00

D H M Woollam (ed) *Advances in teratology* Vol 4 240 pp illus Logos Press Ltd London 1970 100s

U land Forsok till beskrivning av några utvecklings problem SIDA Stockholm 1970

Troubles vasculaires de l'enfant et de l'adolescent Colloque de la Société Française de Phlébologie 172 pp illus Expansion Scientifique Française Paris 1970 F 35

BOOK REVIEWS

H Hejck & G Lundahn *Die progressive Muskeldystrophie und ähnliche Myopathien* Springer Verlag Berlin Heidelberg and New York 1969 436 pp 141 ill DM 118 —

This book gives an excellent review both of historical and of recently discovered facts about muscular dystrophy It is limited to progressive muscular dystrophy other muscle diseases are mentioned only as differential diagnostic possibilities

In chapter 1 the clinical classification of muscular dystrophy and the histopathological findings are discussed The history of the condition is thoroughly reviewed starting with the first known case of muscular dystrophy apparent from an Egyptian picture roughly 3 500 years old One can agree with the authors statement that at present without knowledge of the biochemical background of the various types of muscular dystrophy a classification should preferably be based on genetical grounds and also with their own objection that this is usually impossible in reality Thus their own classification like most other usable classifications is based on a mixture of clinical and genetical features The description of the various types of muscular dystrophy is clear the authors large personal experience with a large material of muscular dystrophy makes this part of the chapter particularly valuable The discussion of the findings on routine histopathological examination of muscle specimens adds little to our previous knowledge

In chapter 2 Elisabeth Freund Molbert gives a beautiful description of the electronmicroscopical findings in muscle diseases

Chapter 3 reviews in an excellent way the known facts about the metabolic disorders present in muscular dystrophy concentrating on the authors main interest the abnormal enzyme activity in blood and muscle tissue The variation of the enzymes according to age and their distribution within the muscle cell and between various muscles in health and disease are described in great detail This work represents a fascinating and important contribution to our basic knowledge about the muscle enzymes The number of enzymes studied is overwhelming One only misses a more profound discussion about the activity in the serum of the isoenzymes of lactodehydrogenase as the examination of these isoenzymes seems in some patients and in some healthy gene carriers to be as important as the study of creatine kinase It should also be pointed out that the whole chapter is concerned only with the biochemical aspects and that histochemical studies of muscle tissue an important contribution to our knowledge of muscle diseases is mentioned only briefly

Chapter 4 is concerned with the distal types of muscular dystrophy chapter 5 with ocular types, chapter 6 with congenital forms and chapter 7 with menopausal myopathies In these chapters the authors review the present knowledge and the controversial opinions

In chapter 8 Herbert Muller-Stephann & Peter Schmidt Peter describe the possibilities of physical therapy and orthopedic measures in muscle diseases One can fully agree with these authors that physical therapy is extremely important to help the patient although it does not cure the disease and that immobilisation will lead to a rapid deterioration A little more debatable is the generous recommendation of various splints as these may cause the patient pain and discomfort and of orthopedic operations to relieve contractures the gain of these operations is not evaluated in relation to the possible loss caused by the necessary immobilisation afterwards.

The discouraging result of all medicamental therapy tried is reviewed in chapter 9 The final chapter is written by Dietrich Tonnis & Manfred Wolter It gives a clear description of the performance of electromyography and interpretation of the findings in adults without adding anything new

This well planned book can be recommended to everybody interested particularly in the clinical and the biochemical aspects of muscular dystrophy and in the possibilities of physical and orthopedic therapy The thorough penetration of the historical background should be particularly stressed the books contains about 1 850 references covering almost 70 pages

Ingrid Gamstorp

D Janz *Die Epilepsien Spezielle Pathologie und Therapie* G Thieme Verlag Stuttgart 1969 554 pp illus., DM 118 —

Dieter Janz's monograph is a milestone in epileptology comparable to the books by Penfield and Jasper and that by William Lennox

Janz dedicates his book to Paul Vogel eminent stylist among German neuropsychiatrists to Hughlings Jackson *the* thinker among *the* ancestors of neurology and to Dostojewsky the explorer of the consciousness of the epileptic

It is evident that Janz himself is an entertaining stylist, a diligent explorer of body and mind and an imaginative thinker

The book is based on vast out patient material that for many years has been subject to versatile investigations by Janz and his many fellow workers at the Heidelberg School

Janz submits a nosographical classification of the *epilepsies that seems convincing and useful* It is based on a meticulous description of the various types of fit and a correlation with the electro-encephalographic findings the age at onset the course of the disease and the influence of the sleeping waking cycle The most clearly defined groups are the five infantile and juvenile forms of petit mal epilepsy The terminology will probably give rise to argument Infantile spasms myoclonic astatic epilepsy and myoclonic jerking are all called petit mal a term which in Scandinavian and English speaking countries is reserved for pyknoleptic petit mal

Each chapter starts with a carefully assembled and very interesting list of synonyms elucidating the scientific *history of epilepsy and preventing the reader* from being confused It also shows as does the whole book Janz s intimate knowledge of the literature

The text is abundant with the evidence of detailed clinical experience Even masters of epileptology will find surprising observations again and again

Faced with controversial problems Janz is not the type to leave them unsolved He preferes to venture upon provocative hypotheses arousing the interest of his reader

A *few* critical remarks

Janz apparently is not fond of statistics Sometimes the reader doubt how safe the basis of the conclusions might be The importance of dosing antiepileptic drugs according to the weight is not mentioned Control of treatment by testing the concentration of the drug in the serum is not touched upon either

The leisurely style and the many repetitions make the book as easily readable as a novel The reviewer *considers this an pedagogic asset*

The reference list is very comprehensive the subject index very differentiated

Janz s book should be read by every epileptologist including every neuropaediatrician The young doctor showing interest in any aspect of epilepsy should be referred *to this book in the first place*

Mogens Lund

N Lloyd Rusby (ed) *New ideas in asthma and its management* The chest and heart association Health Horizon Ltd London 1969 56 pp 10s

Papers read at a symposium in London 1969 over asthma and its management have been collected in a small well worth reading volume In a controlled trial over the effect of Intal (disodium chromoglycate) in pollen allergic asthmatics A W Frankland did not find a significant difference between the control group and the Intal group However he is not negative to the drug we can be certain that further uses will be found for disodium chromoglycate D G Wraith reports results from investigations of dust allergy and mites The observations set forth by the Dutch group (Voorhorst and co workers) that mites from housedust may frequently cause allergic skin reactions are confirmed Some effects of hypnotherapy have been found in asthmatics in a controlled trial by G P Maher Loughnan

Leslie Scott reports preliminary results from a follow up investigation of boys who have passed a Residential Boarding School for asthmatics The group was a highly selected one as the school receives mainly boys who have failed to respond to conventional treatment With this in mind the results must be regarded as *remarkably good 41 of 47 boys* followed up for three years after discharge from the school were leading a normal life or had symptoms which *did not cause interference in the process of* education or earning a living

The inhalant allergens in bronchial asthma in the *U.A.R* have been studied by *A El Hefny* and employment of asthmatics by Helen Zach Two papers concern treatment with bronchodilator aerosols (by *I W B Grant* and *P Heaf*) The title of one of them is Sudden Death in Asthma" Associated with the rise in the death rate there has been a parallel rise in the use of aerosols After the Dunlop Committee working in 1967 and the decline in the sales of these aerosols the mortality has also fallen

Bengt Kjellman

O Koldovsky *Development of the Functions of the Small Intestine in Mammals and Man* 204 pp illus S Karger Basel New York 1969 DM 53—

In the last ten years there has been an increasing interest to study the development of the morphology and function of the gastrointestinal tract The Laboratory of Developmental Nutrition in Prague under dr O Koldovsky is one of the leading centres for this subject In this book dr Koldovsky has in an excellent way summarized his and his collaborators extensive studies in this field and furthermore collected all information available in literature today

The book can be divided in two parts The one deals with the development in different mammals and the other with the human fetus For natural reasons the first part is the most extensive The changes of macro and microstructure of small intestine the development of motor activity as well as the absorption of water ions and vitamins are discussed A relatively large part is devoted to the digestion and absorption of carbohydrates where the existence of several beta galactosidase activities has been especially discussed The development of digestion and absorption of fats has only been studied to a relatively small extent while more information concerning the proteins and amino acids is available One of the most interesting questions in this field is the problem of regulation of protein transfer through the mucosal cells occurring in the neonatal period in several species The developmental changes of other enzyme activities as alkaline phosphatases and beta glucoronidase are also discussed The book ends with a long and apparently complete reference list

knowledge of developmental changes of the function of the small intestine is not only of great physiological interest but it also contributes to our understanding of pathological conditions due to enzyme defects. Therefore this book is highly recommended to the gastroenterologically interested paediatrician.

Tor Lindberg

G E W Wolstenholme & M O'Connor (eds) *Foetal Autonomy*. A Ciba Foundation Symposium. J & A Churchill Ltd London 1969 326 pp 70s

During development the mammalian organism passes through a number of critical periods—at implantation, during organogenesis, at birth and after birth during the establishment of behavioural patterns—periods which pass never to return. Failure to make the transition at the right time is crippling or lethal. The mechanisms concerned are fascinating and difficult to unravel. Investigators separated not only by distance but even more by discipline are working on these problems. With the purpose of exploring different approaches to the study of fetal autonomy a Ciba symposium was held in London in December 1968 under the chairmanship of Dr G S Dawes. The proceedings of this symposium are published in this volume.

R V Short is searching for the answer to the question: How soon does an animal know that it is pregnant? Is implantation an essential prerequisite for the maternal recognition of pregnancy? The human and the monkey embryo implant and secrete a chorionic gonadotropin that may be luteotropic before the first missed menstrual period and thereby causing the first maternal recognition of pregnancy. In the horse however pregnancy may even be recognized while the egg is still in the Fallopian tube. Another intriguing problem considered is the immunological relation between mother and fetus. Why does the pregnant woman tolerate her baby which being an allograft should be incompatible with her and so be rejected? According to C A Currie the mother's immune mechanisms are confronted by fetal antigen from the blastocyst at the time of implantation and later by the trophoblast. The maternal antibodies will localize on any fetal structure within reach. This will be on the trophoblast being the only tissue in direct contact with maternal cells, but as the trophoblast is of low antigenicity it is not destroyed by the antibodies. The antibodies adsorb to the throphoblast surface and inhibit cellular immune reactions both maternal-to-fetal and fetal-to-maternal. Besides that the throphoblast acts as barrier by effectively limiting the number of cells passing between the two circulations.

A number of papers are concerned with the hormonal balance and steroid metabolism in the fetus. The development of the fetal lung in different species is discussed by R E Pattle with interesting aspects on the respiratory distress syndrome of the human newborn, e g the comforting fact that many foals suffering from the "barker" syndrome (i e RDS of foals) may develop into good racehorses. The course and distribution of the fetal circulation and fetal blood gas homeostasis is described in an excellent paper by G S Dawes while H Tuchmann-Duplessis reviews the reactions of the fetus to drugs taken by the mother. The discovery that in the mouse a genetic ataxia can be prevented by giving the pregnant animal a supplement of manganese reveals new fields of research and provides hope of finding new ways to prevent congenital malformations.

The different papers and especially the discussions following each paper presented in this volume should be of great interest to everybody seeking the integrated function of the whole fetus, the mechanisms which regulate it and their limits of tolerance on which fetal autonomy depends.

Nils W Svenningsen

J L Melnick (ed) *Progress in Medical Virology* vol 11 S Karger Basel and New York 1969 469 pp US $21.35

The 1969 issue of Progress in Medical Virology like many previous volumes is not primarily devoted to subjects in the field of paediatrics but still it is of great general interest to everyone working within or on the border of infectious diseases. As usual topics of great current virologic interest have been chosen. Some subjects are mainly of basic virological or methodological interest. So are the informative survey by Darlington & Moss on recent work on the envelope of herpes virus, the interesting paper by Dougherty & Di Stefano on cytotropism of leukaemia viruses and the excellent article by Howe, Morgan & Hsu on recent virologic applications on ferritin conjugates. These three papers are all illustrated with beautiful electron micrographs. Hoorn & Tyrrell summarize the use of organ cultures in virology.

Some articles represent a more clinical approach. These include the interesting survey of Herriott on implications of infectious nucleic acids in disease—clarification in this field may possibly develop in the expanding investigations of "slow viruses". Pereira gives a distinguished review on the antigenic spectrum of influenza. The mechanism of antigenic variation and the origin of virus subtypes is still poorly understood. Further knowledge may be gained i.a. through search for new influenza viruses in animals. Zu Rhein's comprehensive article on the association of a newly recognized papovavirus with a human demyelinating disease (progressive multifocal leukoencephalopathy PML) is of interest to the clinician as is the informative survey by Haughton & Nash on normally occurring transplantation antigens along with data on antigenic changes in neoplasms. The understanding of control of cancer may possibly come from further study of tumor immunology.

Some subjects are of explicit clinical—and also paediatric interest e g the comparison of two attenuated rubella virus vaccines one developed in the

USA and described by *Parkman & Meyer* and one in Belgium and reported by Huygelen Peetermans & Prinzie. Problems in immunization against mumps are discussed by Deinhardt & Shramek. An extraordinary interesting report is published by Hope Simpson & Higgins on a combined field and laboratory study of acute respiratory illnesses occurring during 5 years in their practicepopulation of 3 500 persons. This investigation makes a real contribution to the understanding of ways in which the common respiratory viruses participate in the recurrent pattern of human illnesses. It is somewhat to be regretted that regular serologic investigations and the use of controls were not included in this otherwise excellent study. The work is an outstanding example of the opportunities for investigative work open to the interested physician in general practice.

In a traditional final section of the volume the Editor presents a brief note on the development of the classification of viruses.

Curt Carlström

I. Schulman (ed.) *Advances in pediatrics* vol. 16. Year Book Medical Publishers Inc. Chicago, Ill. 1969. 469 pp. US $15.00.

The 1969 volume of Advances in pediatrics appears under the auspices of a new editor and a new editorial board. Since 1947 this wellknown series has been guided by Dr Samuel Z. Levine; with volume 16 the leadership passes to Dr Irving Schulman. In his preface the new editor expresses the hope that the high standards which have been established during the past 22 years will be maintained. The present volume leaves no doubt that these intentions have been realized. Thirteen articles of a very high quality are presented, about double the average number of preceding volumes, *dealing with diverse subjects of modern* pediatrics. Space prohibits a complete review of the content but it may be mentioned that articles like "Newer viral exanthems" by James D. Cherry and "*Advances in pediatric* ophthalmology" by Max Kaplan in an excellent way summarizes information which for the average pediatrician must be hard to collect from different sources.

C. C. Bergstrand

C. N. Barnard & V. Schrire. Die Chirurgie der Herzmissbildung. Springer Verlag. Berlin, Heidelberg and New York 1969. 152 pp. DM 12.80.

This book is written by the well known heart team Barnard & Schrire and deals with the problems most often met by general pediatricians. Young cardiologists and thoracic surgeons will get good information from this book as it is concentrated and easily read.

The authors discuss the six most common congenital heart diseases (persistent ductus arteriosus, coarctation of the aorta, atrial septal defect, ventricular septal defect, Fallot's tetralogy and pulmonary stenosis with intact ventricular septum).

For every condition a clinical description, relevant investigations, indication for operation and method *of operation are given.*

The book is supplemented with excellent photographic and schematic illustrations.

The preferred age for operation is between 5 and 10 years *for all these congenital diseases as the operative* mortality is lowest at that age. The mortality for PDA is 2.6%. For coarctation of the aorta and secundum atrial septal defect 0. Barnard & Schrire have 35 cases of primum defects with 2 deaths. In 74 cases with VSD 6 patients died and in the cases with pulmonary stenosis 36 had valvular stenosis (? died) and 10 had infundibular stenosis (1 died). The first 100 cases of Fallot were operated upon with a mortality of 12%, this is now lowered to 2%.

These surgical results are as expected excellent and underline the importance of early examination of these children. If the clinical examination gives suspicion of heart disease the procedure is early transferring of the child to a team of cardiologists and cardiac surgeons for further investigation and evaluation for surgery.

I can recommend this book to doctors concerned with the welfare of children.

P. Laurdsen

General Index

Authors Index

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Subject Index